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VOL. 20 DECEMBER, 1934 No. 3

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The Journal of Laboratory and Clinical Medicine

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The Journal of Laboratory and Clinical Medicine

VOL. 20 DECEMBER, 1934 NO. 3

CLINICAL AND EXPERIMENTAL

EFFECTS OF HYPERPYREXIA INDUCED BY PHYSICAL MEANS UPON COMPLEMENT-FIXING ANTIBODIES*

L. G. HADJOPOULOS, M.D., AND WILLIAM BIERMAN, M.D., NEW YORK, N. Y.

THE hyperpyrexia occurring in acute infectious diseases has been considered as playing an important rôle in the production of immune reactions. This idea, however, has not been based upon exact experimental evidence because of the impossibility of separating the antigen factor (bacterial protein) from the consideration of the temperature elevation per se.

Of late years several methods have been developed for the elevation of systemic temperature by physical means. These include hot baths, diathermy, radiant heat (photothermy), and radiothermy (30-meter short wave electromagnetic radiation). We have elevated the systemic temperature in human and in animal subjects by means of radiothermy and then maintained it by means of photothermy. This has given us the opportunity of observing the serologic changes produced by hyperpyrexia uncomplicated by the presence of any extraneous foreign proteins.

The effect of hyperpyrexia on the nonspecific alexin (the natural complement of the blood) and on specific agglutinins (antityphoid) have been sufficiently studied to warrant the drawing of temporary conclusions. It has been shown that the alexin in the serum is lowered following exposure to radiothermy. Similarly, the specific agglutinins induced by the parenteral introduction of typhoid vaccines in rabbits is also reduced, and by repeated exposures this specific titer can be completely lost.[1]

*From the Departments of Bacteriology and Physical Therapy of the Beth Israel Hospital. Received for publication, December 15, 1933.

In our experience, limited to a small number of experiments on rabbits exposed to radiothermy, we observed a definite, though slight, primary stimulation of the complementary titer of the serum followed by a fall to normal and even to subnormal figures. These findings were in conformity with our views on the production and disposal of complement as previously described with reference to malarial fever and to other infections.[2] The immediate effect of radiothermy on typhoid agglutinins was not as conclusive as that on the complement. Although a slight stimulation phase could at times be observed, the after-effects were invariably of the depressing nature, lasting from two to four days. The cumulative depressing effect of repeated exposures, as noted by us, are in accord with observations of other investigators.

As we know little or nothing of the effects of hyperpyrexia on the higher order of immune reactions, such as the production of complement-fixing anti-

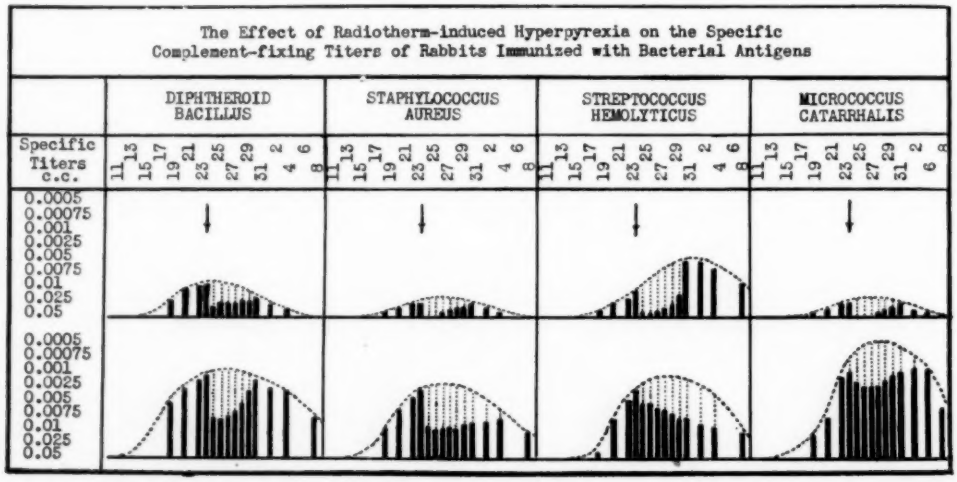

Chart 1.—The graph is drawn on the basis of data given in Table I. The heavy vertical lines stand for the daily titers of the specific complement-fixing antibodies. On the date designated by the arrow (July 24), animals of Series 1 and 3 were exposed to radiation. The course of immunity thereafter, instead of following the hypothetical curve (based on the immunity titers of nonradiated rabbits of Series 2 and 4) was reduced considerably and ran an irregular course. The ground thus lost is marked by the dotted lines.

bodies, we undertook to study this phenomenon in greater detail. Out of a healthy stock of 20 rabbits we kept four as controls. The remaining 16 were divided into 4 groups of 4 each. Each group was then immunized by repeated intravenous injections of one type of bacterial antigen (phenol killed bacteria in saline suspension) and the immunity response was watched closely by means of daily tests beginning on the fifth day following the last administration of antigen.

The bacterial antigens used in these experiments were of the common pyogenic microorganisms; staphylococcus, streptococcus, *micrococcus catarrhalis* and *Diphtheroid bacilli*. The daily titers of complement-fixing antibodies were determined quantitatively in terms of the least amount of serum capable of fixing a unit of complement in the presence of a unit of antigen.

In the course of these experiments we noticed that the stimulation of antibody production in rabbits was not uniform. Identical dosages of the same antigen stimulated some animals to a moderate degree and others to a markedly higher degree. We attributed this peculiar behavior in part to the variations in individual sensitivity inherent in animals in general. Some of the control rabbits showed a natural though very low-grade immunity to bacterial proteins. As the possibility of a latent focus of infection could not be excluded we were inclined to believe that such or similar factors were at

TABLE I*

THE EFFECT OF RADIOTHERM-INDUCED HYPERPYREXIA ON THE SPECIFIC COMPLEMENT-FIXING TITERS OF RABBITS IMMUNIZED WITH BACTERIAL ANTIGENS

SERIES 1 AND 2	*Diphtheroid Bacillus*		*Staphylococcus Aureus*		*Streptococcus Hemolyticus*		*Micrococcus Catarrhalis*	
	R 1	2	R 1	2	R 1	2	R 1	2
	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.
Jul. 11-14	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
19	0.02	0.02	0.04	0.03	0.04	0.04	0.04	0.04
21	0.01	0.015	0.03	0.025	0.025	0.03	0.03	0.03
23	0.009	0.01	0.025	0.02	0.02	0.025	0.025	0.025
24	0.008	0.009	0.025	0.015	0.015	0.025	0.025	0.025
	0.025		0.05		0.04		0.05	
25	0.03	0.009	0.05	0.015	0.045	0.02	0.05	0.025
26	0.025	0.008	0.05	0.01	0.05	0.015	0.05	0.025
27	0.025	0.008	0.04	0.01	0.045	0.01	0.05	0.03
28	0.025	0.009	0.035	0.01	0.03	0.009	0.045	0.035
29	0.02	0.01	0.03	0.01	0.025	0.008	0.035	0.04
30	0.02	0.02	0.03	0.015	0.015	0.0075	0.03	0.045
31	0.02	0.02	0.025	0.02	0.005	0.0075	0.025	0.05
Aug. 2	0.025	0.025	0.03	0.03	0.005	0.009	0.035	0.05
4	0.035	0.04	0.04	0.04	0.006	0.01	0.04	0.05
8	0.05	0.05	0.05	0.05	0.008	0.025	0.05	0.05
Reduction	1/3		1/2		1/3		1/2	
SERIES 3 AND 4	R 3	4	R 3	4	R 3	4	R 3	4
Jul. 11-14	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
19	0.005	0.02	0.009	0.04	0.03	0.04	0.015	0.02
21	0.0025	0.0075	0.006	0.025	0.0075	0.03	0.0075	0.01
23	0.0015	0.005	0.003	0.02	0.004	0.025	0.001	0.007
24	0.001	0.0025	0.0025	0.015	0.0025	0.015	0.0009	0.005
	0.0075		0.008		0.005		0.0015	
25	0.0075	0.002	0.0085	0.015	0.005	0.01	0.0015	0.003
26	0.0075	0.0015	0.009	0.015	0.005	0.009	0.002	0.0025
27	0.007	0.0015	0.009	0.015	0.006	0.008	0.002	0.002
28	0.0065	0.002	0.009	0.02	0.0065	0.0075	0.002	0.0015
29	0.005	0.003	0.009	0.02	0.007	0.0065	0.0015	0.001
30	0.0025	0.005	0.0085	0.025	0.0075	0.006	0.001	0.001
31	0.0015	0.0065	0.0085	0.025	0.0075	0.0055	0.0009	0.001
Aug. 2	0.002	0.01	0.008	0.03	0.008	0.005	0.0008	0.001
4	0.0025	0.03	0.0075	0.04	0.0085	0.005	0.0008	0.0015
8	0.0075	0.04	0.01	0.05	0.01	0.0075	0.004	0.002
Reduction	1/15		1/3		1/2		1/2	

*R as appears in Series 1 and 3 indicates that these animals have been exposed to the radiothermy treatment.

On dates July 11-14 rabbits received the antigenic injections.

On July 24, the first reading indicates the specific titer before exposure to radiothermy, and the second immediately after the completion of treatment.

The specific titers of rabbits of Series 2 and 4 are also given in the table for the sake of comparing the course of immunity production with and without the effect of radiothermy.

In the lines headed Reduction the figures indicate that the specific titers, after radiotherm exposure, have been reduced to these fractions of the original titers before exposure.

the basis of these variations. To minimize the influence of these variable factors we regrouped our animals according to the degree of immunity in them so as to include those with a moderate antibody production in Series 1 and 2, and the remainder showing marked antibody formation, in Series 3 and 4.

To test the effect of hyperpyrexia on the course of complement-fixing antibody formation, rabbits of Series 1 and 3 were exposed to radiothermy for a sufficient length of time to raise their temperatures to 41-42° C. After these temperatures were attained they were maintained for three to four hours by exposing the animals to carbon filament electric lamps. The animals were bled immediately before and at the completion of the experiments and thereafter daily to observe the whole immunity phase. Animals of Series 2 and 4 were kept as controls to Series 1 and 3 so as to permit the determination of the immunity curves with and without radiothermy. The results of these experiments are given in tabular and graphic forms (Table I and Chart 1).

The reduction in the complement-fixing titer of the serum after exposure to hyperpyrexia is due, in all probability, to a slowing up of the production of antibody rather than to its destruction. By observing the daily fluctuations in the antibody titers of treated rabbits we could note a delayed stimulation phase in the majority of these animals.

CONCLUSIONS

The production of hyperpyrexia by physical means causes a temporary diminution of the complement-fixing antibody titer of rabbits immunized against staphylococci, streptococci, *Micrococci catarrhalis* and *Diphtheroid bacilli.*

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A CASE OF MERCURIC CHLORIDE POISONING TREATED BY EXSANGUINATION-TRANSFUSION*

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HISTORY

ALTHOUGH the Egyptians as early as 1600 B.C. were acquainted with the use of mercury, they did not use it as a medicine, and Aristotle (384-322 B.C.) alone of the early Greeks prescribed it for certain skin conditions, as it was generally regarded too poisonous for internal use. However, it was used in India as a medicine possibly as early as 500 B.C. and old records show that mercurial gingivitis was known there as an entity. From India the knowledge of the medicinal value of mercury spread to Persia, and thence to Arabia; and after the birth of Christ it came into high repute as a universal panacea. Mesuë the Older (857) first carefully described mercury inunctions for skin disorders. About this time Arabian culture was rising above the European, and Avicenna and Constantinus Africanus described the toxic effects of mercury as pasty sores in the throat, bad breath, cramps, blood in the stools, disturbances of hearing and vision, and other of the familiar symptoms.

With the mingling of Arabian and European science through the Crusades, the method of mercury inunction gained root, even in the stronghold of Hippocratic teaching at Salerno, and during the Middle Ages came the development of the "salivation cures." Guy de Chauliac recognized the connection between mercury intoxication and salivation, but regarded it as a sign that the treatment was effective, since withdrawal of phlegm was considered as highly beneficial in gout. The Europeans found that mercury given either per os or by inunctions could cause diarrhea, thirst, anuria, and loss of appetite. Disturbances of vision, hearing, and speech were also noted.

Theories and speculations as to the action of mercury within the body were numerous and Thomas Sydenham (1624-1689) declared without doubt that mercury cured syphilis through salivation and that two to three liters of saliva was the proper amount for the patient to secrete each day. This type of treatment gave plenty of opportunity for the observation of mercury poisoning, and Jean Astruc of Montpellier divided the therapeutic or salivation stage from the intoxication stage; the latter, from his description of bloody stools, swelling of the face and bloody sputum, must have been near death from overtreatment.

John Hunter (1728-1793) declared that the toxic effects of mercury combined with the degenerative power of syphilis to act in double force upon the body, and this idea became enlarged upon until by the beginning of the nineteenth century, the symptoms of mercury poisoning and syphilis were

*From the Department of Internal Medicine, University of Kansas.
Received for publication, March 8, 1934.

much confused. A book was published in 1839 by J. P. Murphy showing how mercury alone caused the symptoms usually described as secondary syphilis.

It remained for Robert Overbeck in 1861 to show by clinical and experimental study the true nature of mercury poisoning. In the same year Kussmaul published a report showing the difference between chronic hydrargyrism and constitutional syphilis. These two articles were the chief points in dispelling the many theories and confusion about mercury poisoning.[1]

TREATMENT

The last fifteen years have seen the treatment of mercury poisoning, particularly mercuric chloride, advance from questionable therapeutic measures prescribed by rote and usually without hope for the patient, to a sane, rational procedure, arrived at by experiment and observation, with a gratifying decrease in mortality. Before 1910, mercuric chloride poisoning was considered to be almost universally fatal, but Weiss[2] in 1924 reported 135 cases treated by the newer methods with but 8 deaths. Other men have reported equally good results, so it is apparent that considerable advance has been made in treating these cases.

The former conception has been that albuminous materials when combined with mercuric chloride rendered the poison insoluble and nontoxic; but this is not the case, for mercury albuminate is equally as toxic as the chloride. In fact any ingested mercury is first converted into the albuminate before it is absorbed, the difference being that it is not corrosive as is the mercuric chloride. Accordingly, it is to be remembered that the administration of albumin is to be followed by as prompt an evacuation of the stomach contents as possible.

The next step in treatment was to attempt to neutralize the absorbed mercury. Calcium sulphide was given enthusiastic acclaim in 1917 by Wilms[10] who found it gave excellent results if a freshly prepared solution was given intravenously within twenty-four hours after ingestion of poison. Ravaut[3] in 1920 described the use of sodium thiosulphate in treating arsenical dermatitis, and McBride and Dennie[4] popularized the use of this drug in converting toxic metals into nontoxic sulphides. Although Melville and Bruger[5] concluded from animal experimentation that sodium thiosulphate did not combine with mercury within the body, it remains the opinion of many men that it is of much benefit in treating mercuric chloride poisoning. Marchbanks, Church, and Smith[6] reported a case in 1931 in which the entire credit for saving the patient's life was ascribed to sodium thiosulphate given intravenously.

The Lambert routine of treatment aiming at elimination, alkalinization, and diuresis, has met with considerable favor and while not as popular as ten years ago, still has a number of valuable points. Modifications of this treatment were reported by Weiss[7] in 1917 and Rosenbloom[8] in 1919 with excellent results. Lemierre, Laudat, and La Porte[9] believed that sodium chloride in isotonic and hypertonic solution intravenously resulted in amelioration of symptoms and favored renal excretion. They reported two cases.

Surgery has been used in treating these cases for many years. Anderson[11] tried irrigating the colon through a cecostomy opening. He reported a case with recovery in which the operation was done fourteen hours after ingestion of the poison. Decapsulation of the kidneys has also been done but no longer holds much favor. More recently Berger and others[12] reported a series of cases in which it was shown that immediate cecostomy and constant colonic lavage was the best method of preventing and treating the gangrenous colitis of the mercuric chloride poisoning.

REPORT OF CASE

The case here reported is different from others in that the chief mainstay in the method of treatment is one to which we are unable to find any reference in the literature as having been used in human beings, namely exsanguination-transfusion. It was done for two chief reasons: first, as shown by Rosenbloom[13] and others, the blood and other tissue juices contain the greater part of the absorbed mercury, so by replacing the blood the poison could be removed in the largest possible quantity. Second, by replacing the blood in sufficient quantity, the nitrogenous products retained in the body would be diluted and kept at a sublethal level until the renal epithelium might regenerate. The same method has been of service in delaying uremic death in cases of chronic nephritis.

In 1915, Robertson[14] proposed exsanguination followed by transfusion in cases of severe bacterial toxemia and believed that it might be of value in treating chemical poisoning as well. Haskell and others[15] working with dogs (six in all) and using the minimum lethal dose of mercuric chloride as found by Sansum[16] bled the animals immediately after intravenous administration of the poison and transfused with matched blood. In spite of repeating the procedure, all the dogs died within an average time of twenty-four hours and the authors were of the opinion that the exsanguination-transfusion method of treating mercuric chloride poisoning was of no value. In 1917, Burmeister[17] showed by the same method that although the animals died, the degeneration of the kidney epithelium in dogs and rabbits was distinctly inhibited by the use of transfusions.

In our case in addition to the bleeding followed by transfusions, the patient was given a small amount of sodium thiosulphate for its reported value in neutralizing available mercury. Sodium chloride was given to combat loss of chlorides by vomiting.

M. C., white male, aged twenty-three, entered Bell Memorial Hospital May 22, 1933, with a chief complaint of inability to get his breath. Eight days previously in the evening he had swallowed 18 gr. (1.3 gm.) of mercuric chloride with suicidal intent. It was nearly an hour later before he vomited. He continued to vomit and choke for the remainder of the night. He was not seen by a physician until the following morning. The treatment for the eight days previous to hospital admission consisted of fluids, principally milk, and daily intravenous injections of sodium thiosulphate. He said that on the

third day he began getting short of breath but did not notice scanty urine until the following day. Bloody diarrhea was severe before admission to the hospital.

On admission he was gasping for breath, and his skin was bluish, dry, and cold. His breath was fetid. Blood oozed from the soft, swollen, inflamed gums, and there were hemorrhagic areas on the uvula. Moist râles were heard in the base of each lung. Examination of the heart revealed a rate of 110, blood pressure, systolic 170, diastolic 100, and there was redupli-

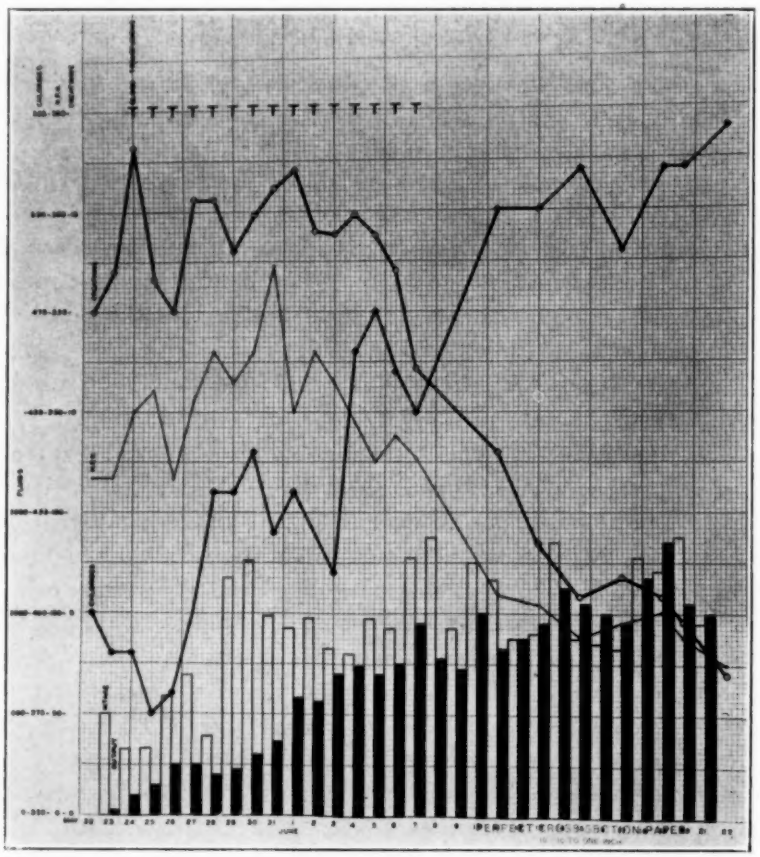

Fig. 1.

cation of the second mitral sound. The abdomen was distended and tender. The liver was palpated 10 cm. below the costal margin and its edge was rounded and tender. On admission the blood chemistry showed per 100 c.c.: sugar 83 mg., nonprotein nitrogen 167 mg., creatinine 12.5 mg., chlorides 400 mg. Urine showed a specific gravity of 1.014, strongly alkaline, trace of sugar, albumin two-plus, many hyaline and granular casts. Hemoglobin was 70 per cent, R.B.C. 3,160,000, W.B.C. 20,500, with 89 per cent polymorphnuclears.

He was given fluids by mouth, 50 c.c. of 50 per cent glucose intravenously, and 15 gr. of sodium thiosulphate intravenously in the first twenty-four hours.

The patient seemed in about the same condition at the beginning of his second hospital day, but toward the end of this day he had more respiratory difficulty, developed auricular fibrillation, the cyanosis increased, and the end seemed to be near at hand. At this time it was decided to bleed and transfuse the patient. A list of 25 suitable donors was compiled and in the evening of May 24 (forty-eight hours after admission), 850 c.c. of blood was withdrawn and 1,000 c.c. of citrated blood given by indirect method. For the next two weeks the patient was bled an average of 700 c.c. and transfused 800 c.c. daily. On the third and fourth hospital days the patient seemed to be getting worse. His pulse became weak, the abdomen distended, and blood flowed freely from the gums. He rallied somewhat at the end of the fourth day and showed improvement, gradual at first, then more rapid as the urinary output began to increase. Fifteen grains of sodium thiosulphate were given intravenously daily for the first three days of hospitalization. Sodium chloride in 45 gr. dosages was given daily for the first four days, when vomiting was severe. There was no profuse diarrhea during the time he was in the hospital, but blood was recovered from the stools. After 15 daily transfusions were given they were discontinued because of clinical improvement in patient and the gradual increase in urine output.

At the time of dismissal, the gums were healed; the heart was regular and pulse normal; there was no dyspnea on exertion; no occult blood in the stools and the urinary output was normal. The blood chemistry records and fluid intake and urine output during the stay in the hospital are shown in Fig. 1.

The patient was seen seven months after dismissal (Jan. 22, 1934), when examination showed urine entirely negative. Blood pressure, systolic 140, diastolic, 80. Blood chemistry: nonprotein nitrogen 35.3, creatinine 1.6, urea 11.81, sugar 82, and chlorides 460. Clinically he was a well man.

CONCLUSIONS

1. Severe mercury poisoning is treated successfully by a new method, that of exsanguination and blood transfusion.

2. Sodium thiosulphate intravenously had seemingly proved unsuccessful in the treatment of this case.

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NODULAR GRANULOMATOUS LESIONS OF THE LIVER, SPLEEN, AND LYMPH NODES

PROBABLY AN ATYPICAL FORM OF HODGKIN'S DISEASE*

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IN A recent paper, Beaver and Johnson[1] have described the case of a young woman who died following a typhoid-like illness associated with fever, marked abdominal distention, diarrhea, and terminal coma. The striking nodular, necrotic lesions in the liver and spleen of this patient were at first thought to represent a form of tularemia, but further study convinced the writers that they were dealing with an unusual type of Hodgkin's granuloma. Since no corresponding example of this disorder has to our knowledge been reported in recent literature, it seems desirable to report a second case in which the terminal clinical picture resembled that of portal cirrhosis, and in which similar nodular granulomatous lesions of the liver, spleen, and lymph nodes were found at necropsy.

While neither the first case nor that presented in this report can as yet be accurately classified on either a clinical or pathologic basis, they are reported in the hope that other observers may be able to correlate them with similar cases and thus clarify their position in respect to other and better recognized granulomatous processes.

REPORT OF A CASE

A housewife, aged twenty-seven years, first registered at The Mayo Clinic in July, 1924, complaining of swelling of the left side of the neck. The past history was irrelevant, except for many attacks of tonsillitis in early life. Five years before, tonsillectomy and removal

*From the Mayo Clinic.
Submitted for publication March 20, 1934.

of the lymph nodes of the left side of the neck had been performed elsewhere. Details as to the histologic appearance of these nodes were not available, but the patient assured us that they had been regarded as tuberculous.

On physical examination the patient was in good general condition except for a mass of firm, discrete lymph nodes in the left cervical region. A single enlarged node was also noted in the right axilla. These nodes varied from 0.5 to 4 cm. in diameter, the largest being situated directly behind the angle of the jaw. Laboratory examinations including roentgenograms of the thorax, urinalysis, blood count, and Wassermann test of the blood, gave negative results. The patient did not wish to have a node excised for biopsy and, consequently, radium treatment was advised. A total of 3,000 mg. hours of radium was applied to the left submaxillary and upper and middle cervical areas, and 2,000 mg. hours to the right axilla. Two months later another course of radium treatment was given.

The patient's second admission to the clinic was in November, 1925. She stated that the nodes previously described had been greatly reduced in size after treatment with radium, but that in the summer of 1925 they had become enlarged again and the node in the right axilla had caused her some pain. Results of physical and laboratory examinations were essentially the same as at the time of her previous examination. A third course of 5,000 mg. hours of radium was given over the involved nodes with satisfactory results.

The patient's third examination was in July, 1932. During the preceding seven years the nodes had alternately increased and decreased in size, but she had had no general symptoms until shortly before admission, when loss of weight, nausea, and vague indigestion had been noted. On physical examination the same mass of nodes that was present eight years previously was noted in the left cervical region, extending from the mastoid region to the clavicle. Physical examination was otherwise not remarkable except for definite enlargement of the liver which was palpable about 4 cm. below the right costal margin. The tip of the spleen could also be felt. A careful search was made for a distant tuberculous focus, but nothing definite could be demonstrated. Roentgenographic examination disclosed slight widening of the mediastinum, but otherwise laboratory examinations gave negative results. Because of the unusual persistence of this cervical adenopathy, the patient was again advised to have a node excised for biopsy. This she declined to have done and asked that another course of radiotherapy be given. Because of the questionable mediastinal shadow, the mediastinum was included in this course of treatment. As on previous occasions, radiotherapy caused progressive decrease in the size of the nodes, but she failed to gain in strength. About three months after the completion of treatment she had an attack of herpes zoster, limited to the left lower portion of the trunk, and following this she was almost disabled and was confined to her bed. At this time two new developments were noted: slight fever in the afternoon and progressive increase in the size of the abdomen. The latter was associated with pain in the right upper quadrant, which varied from vague discomfort to actual distress requiring codeine for relief. By December, 1932, a slight hacking cough with a small amount of blood-tinged sputum had developed, and there was also definite edema of the lower extremities.

The patient was readmitted to the clinic in January, 1933, as an emergency case. On physical examination the striking findings were marked pallor, an extreme degree of ascites, and moderate peripheral edema. The cervical nodes were much reduced in size and could be felt only as discrete, pea-sized masses. The thorax was essentially normal, except for an elevated diaphragm and evidence of congestion at the bases of both lungs. Pelvic and rectal examinations, while somewhat unsatisfactory because of the ascites, revealed no findings of any importance. Urinalyses were repeatedly negative, except for an occasional hyaline cast and faint traces of albumin. The value for hemoglobin was 10.5 gm. per 100 c.c. of blood, erythrocytes numbered 3,220,000 and leucocytes 3,400 per cubic millimeter of blood: The differential count was approximately normal, and the blood smears revealed active regeneration of erythrocytes and moderate polychromatophilia. The concentration of bilirubin in the serum was 2.9 mg. and the reaction direct. The value for urea was 18 mg. per 100 c.c. of blood. Roentgenographic examination of the thorax and fluoroscopic examination of the stomach gave negative results. Electrocardiograms showed diphasic T-waves in derivations

1, 2, and 3, but were otherwise normal. A test of hepatic function with bromsulphalein revealed dye retention Graded 4. A tentative diagnosis of portal obstruction with ascites was made, the most likely cause being regarded as portal cirrhosis with secondary splenomegaly. Treatment with diuretics was begun, but the patient reacted so poorly that it was necessary to perform paracentesis on the sixth day of her stay in the hospital. At that time 6,000 c.c. of dark, amber-colored fluid were removed. Cultures were made, but no growth of organisms was obtained. Stained smears from the ascitic fluid were negative for *Mycobacterium tuberculosis* and other organisms. Following this paracentesis, no improvement was noted. The abdominal and pelvic organs were examined carefully after the removal of the ascitic fluid, but nothing could be made out other than the enlargement of the liver and spleen previously mentioned. Throughout the patient's stay in the hospital she had more or less continuous elevation of temperature, which sometimes reached 102° F. Much of the time she was somewhat drowsy and dull, but she was never definitely disoriented. Because of the probability of impending hepatic insufficiency, a liter of 10 per cent solution of glucose was given intravenously daily. In spite of all attempts to produce diuresis, the ascitic fluid slowly reaccumulated, and it was obvious that another paracentesis would soon have to be performed.

Because of the possibility of peritoneal tuberculosis, it was decided to explore the abdomen through a small incision and examine the liver, spleen, and peritoneal surfaces. This was done January 19 by H. K. Gray, the exploration being carried out through a right rectus incision. The omentum and peritoneal surface appeared entirely normal. The hepatic surface was irregular and roughened, suggesting an atrophic type of cirrhosis. The spleen was about six times the normal size and presented no unusual features. The pelvic organs were examined, but no evidence of pelvic tuberculosis could be demonstrated. Talma-Morison omentopexy was performed, and the abdomen closed in the usual manner. The patient failed rapidly after operation, lapsed into coma and died on the fourth postoperative day with signs of hepatic insufficiency.

Necropsy was performed three and a half hours after death. The body was unembalmed. The ankles were edematous, Graded 2. The recent surgical wound in the right upper portion of the abdomen was clean. The peritoneal cavity contained 3,000 c.c. of clear straw-colored fluid; otherwise the peritoneum was normal in appearance. The right pleural cavity was practically obliterated by fibrous adhesions and contained no excess fluid. In the left cavity there were no adhesions, but 150 c.c. of serosanguineous fluid was present. The lungs were essentially normal except for atelectasis of the lower lobes and the presence of calcified tubercles in the right middle and left lower lobes with fibrocaseous tuberculous involvement of the hilar lymph nodes. The pericardial sac contained 65 c.c. of clear straw-colored fluid. The heart was normal in appearance.

The spleen weighed 557 gm. Multiple dark red, grayish pink, or entirely gray nodules of increased consistency were distributed quite generally beneath the capsule and throughout the splenic pulp. These varied in size from 0.5 to 2.5 cm. in diameter and frequently were spherical or irregular in shape and fairly well circumscribed; often, they were slightly raised as rounded nodules when present beneath the capsule. The appearance of some was similar to that of infarction.

The liver weighed 2,533 gm. The consistency was somewhat decreased. The color was light reddish yellow with dark red or grayish patches mottling the surface. The capsule was smooth and glistening and not thickened. The dark red and gray areas rose above the surface slightly as rounded elevations, and measured 1 mm. to 2 cm. in irregular diameter. The appearance of the

sectioned surface was similar to that of the capsular portion. Throughout all parts of the liver the hemorrhagic areas were prominent. They were slightly firmer than the remainder of the liver and devoid of the lobular markings that were well revealed elsewhere. This peculiar mottling of the liver was quite dissimilar to that seen in chronic passive congestion, since the hemorrhagic zones were very irregular in size, shape, and in distribution, and appeared not to be confined to central lobular positions, but, on the contrary, seemed to involve entire lobules or groups of lobules.

Lymph nodes surrounding the tail and head of the pancreas, the porta hepatis, and in the right axilla, were enlarged, of slightly increased consistency, and glistening, grayish white to pink in color with irregularly mottled deep red zones similar in appearance to the nodules in the spleen. Lymph nodes elsewhere were somewhat enlarged and of slightly increased consistency, but, generally speaking, there was nothing especially remarkable about them.

The entire gastrointestinal tract, pancreas, kidneys, suprarenal glands, ureters, bladder, uterus and adnexa, thymus and thyroid glands were normal. The sclerosis of the aorta was Graded 1. The brain was not examined.

In microscopic preparations* of the relatively normal portion of the liver, which constituted less than one-half of the hepatic bulk, there was moderately severe fatty change. Hepatic cells not so affected were usually faintly stained. Central vein areas were irregularly congested, with sometimes slight atrophy and necrosis of central hepatic cords. Throughout all portions of the liver there were cellular collections consisting of lymphocytes and a few endothelial cells. The cellular collections, when small, were confined to the periportal connective tissue, but when larger, they extended through the peripheral portions of the lobules or involved zones as large as, or larger than, a lobular unit. When the aggregations of cells were larger, they were accompanied by areas of necrosis (Fig. 1 A) of varying size, always surrounded by lymphocytes. There was no sign of rapid progression of the necrosis to surrounding hepatic parenchyma, for the hepatic cells near the periphery of these areas were as normal in appearance as those anywhere in the liver. The areas of necrosis were sometimes devoid of all cellular detail, but this varied, for in some, congested sinusoids and reticular cells persisted. In other places there was hyperplasia of reticulum. The cells which we have called "lymphocytes" were morphologically like mature lymphocytes, though perhaps slightly larger. The nuclei were large hyperchromatic structures, and the chromatin was often finely divided. There was a scant amount of pale-staining cytoplasm about some cells, although usually none could be identified. The cells described as endothelial were large, with large, pale nuclei and with abundant cytoplasm, sometimes pale and sometimes intensely acidophilic. A few of these cells were phagocytic for erythrocytes and cellular débris. Occasionally these cells had giant nuclei, and a few were multinucleated, the latter being usually hyperchromatic. The reticular cells were usually spindle-shaped or stellate, sometimes with fine fibrillae at the poles of the cells. Mitotic figures were not identified in any of these cells.

*Tissues fixed in Orth's fluid, embedded in paraffin, and stained with hematoxylin and eosin.

Microscopic sections of the spleen, taken through the red nodular areas, disclosed intense sinusoidal congestion. There were relatively few lymphocytes in the pulp, and malpighian corpuscles were small. In areas of less intense congestion, or at the periphery of the fairly sharply circumscribed hemorrhagic areas, there were proliferated reticular cells and collections of lymphocytes. The lymphocytes were sometimes collected in small aggregations with the hyperplastic reticulum, but often they were independent. The reticular cells were arranged as bands or, in many places, small foci of cells. Sometimes there were small zones of necrosis, surrounded by lymphocytes. Many of the small malpighian follicles of these areas contained masses of hyaline-fibrinoid material. Sections of the white nodular areas revealed extensive necrosis, as in infarction;

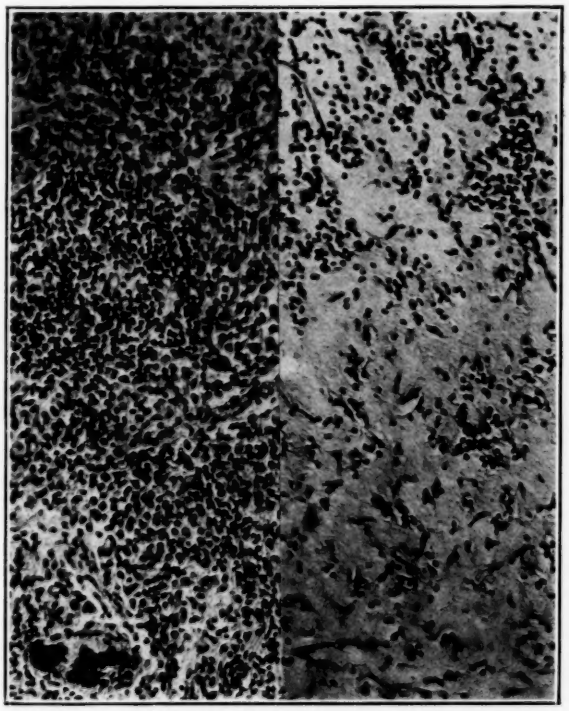
A. *B.*

Fig. 1.—*A*, Liver: two foci composed of lymphocytes and a few endothelial cells. In the lower lesion there is also proliferation of reticulum and necrosis. The upper lesion is small and confined to a periportal space. Extensive fatty changes exist in the hepatic cells (hematoxylin and eosin ×90). *B*, Spleen: A splenic trabeculum with foci of lymphocytes, also perivascular arrangement of lymphocytes (hematoxylin and eosin ×90).

only a few well-stained lymphocytes were present. At the periphery of these areas there was a certain amount of blending with surrounding splenic pulp. In the places of transition, there were hyperplastic reticular cells and foci of lymphocytes. The lymphocytic collections were particularly evident in the connective tissue of the trabeculae, in the walls of larger vessels and surrounding them (Fig. 1 B). In the dense connective tissue the lymphocytes tended toward columnar arrangement.

Lymph nodes of the splenic and hepatic hilum and in the head and tail of the pancreas usually disclosed complete loss of normal nodal architecture. In

the nodes which revealed only mild involvement there was only hyperplasia of the reticulum with some atrophy of lymph follicles. Lymph sinuses were often engorged with endothelial cells or obliterated by hyperplasia of the reticulum (Fig. 2 A). In nodes with extreme involvement neither lymph follicles nor sinuses could be identified and, in addition to areas of reticulo-endothelial cell hyperplasia and dense foci of lymphocytes, there were zones of necrosis apparently in the centers of dense foci of lymphocytes. There were also other large relatively acellular areas. These were well stained with eosin and by careful observation were seen to have a fibrillar or collagenous fibrous structure with few stellate or spindle nuclei and a few irregularly distributed lymphocytes (Fig. 2 B). At the periphery of these areas dense collections of lymphocytes

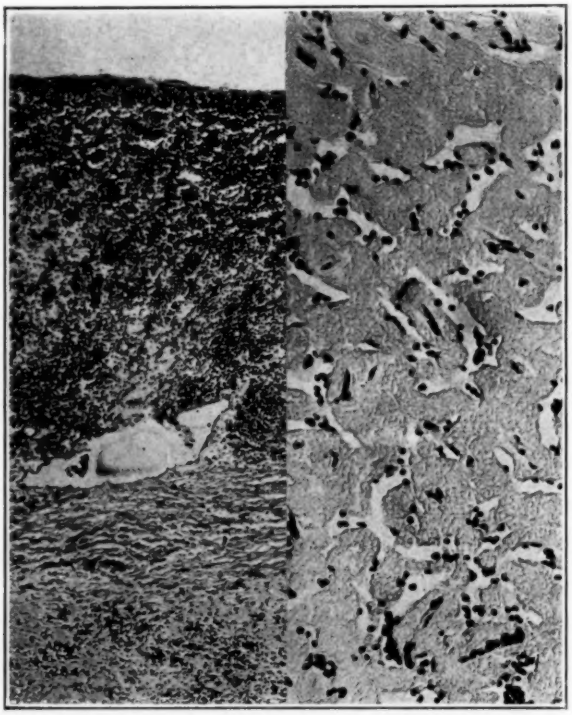


A. *B.*

Fig. 2.—*A*, Mesenteric lymph node revealing loss of lymphoid follicles, engorgement of sinuses by large endothelial cells, and proliferation of reticulum (hematoxylin and eosin ×150). *B*, Pancreatic lymph node disclosing loss of lymphoid follicular architecture, proliferation of reticulum with fibrillar and collagenous connective tissue changes (hematoxylin and eosin ×150).

intermingled with a few larger endothelial cells were especially prominent. The capsules of the nodes were often densely infiltrated with lymphocytes and a few endothelial cells (Fig. 3 A). The lymphocytes and endothelial cells appeared to be mature and did not contain mitotic figures. Sections of mesenteric lymph nodes were similar to those of the pancreatic group that revealed the milder changes. There was focal hyperplasia of reticulum, and sinuses were choked with large endothelial cells. Capsules of the nodes were infiltrated with lymphocytes. Sections of cervical nodes revealed complete loss of follicle structure, reduction of lymphoid elements and sinuses, hyperplasia of reticulo-endothelial

cells, and early fibrosis. There were also many dilated congested blood vessels within the nodes. The capsules were thickened and contained much collagenous fibrous connective tissue. One peribronchial lymph node disclosed a caseous and calcified tubercle without apparent activity. In others there was loss of normal follicular structure. Sinuses were engorged with endothelial cells and lymphocytes and there were areas of marked proliferation of reticulum and fibrosis. Capsules were densely infiltrated with lymphocytes and a few endothelial cells. There were also areas of relatively acellular hyalin deposits containing but few spindle cells and lymphocytes. A lymph node from the right axilla disclosed complete loss of follicular and sinusoidal architecture and replacement by wide



A. *B.*

Fig. 3.—*A*, Pancreatic lymph node and capsule; peripheral nodal lymph sinuses are entirely obliterated; capsule is diffusely infiltrated with lymphocytes and endothelial cells (hematoxylin and eosin ×55). *B*, Axillary lymph node; revealing wide bands of collagenous connective tissue in which a few lymphocytes and stellate and spindle-shaped cells may be seen. The entire node presented this appearance (hematoxylin and eosin ×175).

collagenous fibrils often arranged in wavy bands. With the hyalin there were spindle-shaped and stellate contracted nuclei and a few lymphocytes arranged in columns (Fig. 3 B). Lymphocytes were more numerous near the capsule, which was thickened and composed of dense fibrous connective tissue of collagenous type and contained a few collections of lymphocytes and endothelial cells. A lymph node from the lesser curvature of the stomach was identical in appearance with the node from the right axilla. The morphologic characteristics of the various cells described in the spleen and lymph nodes were identical with those previously described in the liver.

Sections of the lungs were normal except for a healed tubercle in the right middle and in the left lower lobes. The tubercles were fibrocaseous with calcification and without evident activity. In one of the tubercles there was deposition of bone and bone marrow formation.

The microscopic preparations of the left suprarenal gland revealed lymphocytic collections in the medulla. The capsule and surrounding areolar tissues were densely infiltrated with lymphocytes and a few larger endothelial cells. There were areas of necrosis and other places of marked fibrosis. In the areas of fibrosis the lymphocytes were often in dense masses or in columns. Lymphocytes were always mature in type, so far as could be determined. Mitotic figures were never found. Endothelial cells similarly were apparently mature.

Cultures of the blood, spleen, and liver were taken postmortem. They were prepared by inoculating material of the three types mentioned, on dextrose brain broth, and on hormone agar with sterile human blood added to the liver and spleen cultures. Incubation was carried on at 37° C. under aerobic and anaerobic conditions, over a period of three weeks. A few colonies of *Staphylococcus aureus* developed from each cubic centimeter of the patient's blood and from preparations of the liver and spleen. Three guinea pigs were inoculated intraperitoneally, and three subcutaneously with liberal amounts of the patient's blood and with triturated suspension of liver and spleen. Two rabbits were inoculated intravenously with triturated suspensions of liver and spleen. The animals were observed for a period of sixty days, temperatures of the guinea pigs being taken daily the first two weeks, and at no time did they reveal evidence of reaction. Finally all were killed and carefully examined. There were no lesions observable in any of them. Serum from the patient's blood was tested for agglutinins of *Brucella abortus*, *Pasteurella tularensis*, *Pfeifferella mallei* and *Proteus X19* with negative results. Sections of spleen, liver, and lymph nodes were stained by Brown-Gram stain and carbolfuchsin acid-fast stain, without revealing anything except a few micrococci in certain lymph nodes.

COMMENT

The principal clinical features of this case were: first, the long-continued enlargement of the chain of cervical lymph nodes which had repeatedly diminished in size following radiotherapy, and second, the development of hepatic and splenic enlargement, followed by ascites, low-grade fever, and evidence of hepatic insufficiency as shown by clinical signs and also by tests of hepatic function. The latter findings hardly seemed compatible with the diagnosis of tuberculosis, which had previously been made on the basis of a biopsy of a cervical node. For this reason, the terminal phenomena were looked upon as independent of the previous adenopathy, an error which was apparent only at necropsy.

Just as the clinical features of this patient's illness presented complex problems in differential diagnosis, so also did the findings at necropsy. The only evidence of tuberculosis that could be found postmortem was in the form of primary healed foci in the lungs and peribronchial lymph nodes. The diagnosis of tuberculous cervical lymphadenitis was not confirmed by necropsy and, as a matter of interest, the cervical lymph nodes, so far as could be observed, were not remarkable in any respect. They were discrete, of the size of a pea or

smaller, and somewhat increased in consistency. Search was made in the axillary fossae for enlarged nodes because the history of the patient's illness indicated that involvement of lymph nodes in the right axilla had been present. Only one enlarged node, approximately 1 cm. in diameter, was found, this being very firm and grayish white. The clinical and roentgenologic evidence of widening of the mediastinal shadow was not confirmed. Aside from the one node involved by calcified tuberculosis, as already mentioned, there was nothing noteworthy grossly in this region. The really prominent lesions, those apparently responsible for the patient's demise, were confined to the abdominal cavity where there were found ascites, extensive lesions of the liver and spleen and of the lymph nodes composing the lymphatic chain across the abdomen between liver and spleen. The splenic and hepatic lesions grossly resembled multiple foci of neoplastic tissue, such as one might expect in certain types of sarcoma. Some of the lymph nodes were very large and formed masses along the pancreas which were composed of individually discrete nodes, conglomerate only because of loosely adherent intervening areolar tissue. Although not entirely typical, these nodes resembled grossly those seen in lymphosarcoma or Hodgkin's disease.

The microscopic preparations of the various lesions were even more difficult to evaluate accurately. From gross examination it had been anticipated that the lesions would be revealed microscopically as clearly defined Hodgkin's disease or sarcoma. But in contrast to this we found a rather indefinite granulomatous process with lymphocytic collections and extensive focal areas of necrosis in the liver and spleen. In the lymph nodes, there were foci of lymphocytes, hyperplasia of endothelial and reticular cells and zones of necrosis or fibrosis, with loss of sinus architecture and lymph follicular structure. The lesions in the cervical, peribronchial, axillary, splenic, pancreatic, hepatic, gastric, and mesenteric lymph nodes seemed to be of the same fundamental type, with differences only a matter of degree or age. In the liver and spleen, too, the lesions appeared to be pathogenetically related to those of the lymph nodes, representing more severe or earlier manifestations of the same process.

Such changes could hardly be interpreted as neoplastic in the usual sense. They were quite evidently an unusual manifestation of some unknown infectious agent that had produced in the tissues granulomatous reactions of nonspecific type, or at least of a type not to be identified with the granulomatous diseases of known etiology. Not only did morphologic study of the lesions eliminate from consideration the usual forms of specific granulomatous disease, but this had also been done by tissue stains for microorganisms, bacteriologic, serologic, and animal inoculation studies. Etiologic agents of the easily proved infectious diseases were thus eliminated, as well as tuberculosis (mammalian and avian), glanders, syphilis, tularemia, undulant fever, spirochetal diseases of the liver and the mycotic and protozoan diseases.

The presence of *Staphylococcus aureus* as found in the lesions postmortem could hardly be regarded as significant. They often have been found associated with the lesions in Hodgkin's disease; however, they are generally regarded as secondary invaders. In this connection Cunningham stated that: "Staphylococci and streptococci may give rise to a picture simulating Hodgkin's disease histologically, either by direct infection or as an irritative reaction." It had

been our opinion that the staphylococci in our case were secondary or terminal invaders and had in no way been connected with the evolution of the lesions, though no doubt final disposition of this question should be held in abeyance. Wallhauser considers, bacteriologically, that Hodgkin's disease presents "confusing and variegated findings, none of them generally accepted as etiologic."

It is apparent then, that in spite of ordinarily adequate study, the specific etiologic agent responsible for the lesions in our case had missed detection. Of the granulomatous diseases with which our case might be classified, that is, granulomas with unknown etiology, primary consideration must be given to Hodgkin's disease. In retrospect, the history of the case would coincide with such a diagnosis, and the findings, and at necropsy the morphologic studies, although not classical, could be considered sufficiently characteristic to admit this diagnosis. The possibility must be considered that in our case the unusual picture had been evolved by some modifying agent, such as might be produced by radium and secondary infection, also, that the disease existed in two phases, both of which are sometimes difficult to diagnose, the first being the latent or fibrotic stage and the second the earliest or acute phase. Cunningham stated that: "The earliest pictures of Hodgkin's disease are like those of any infection or irritation, a hyperplasia of lymphoid elements, and though the disease be suspected clinically, a histologic diagnosis cannot be made, although probable or suspected Hodgkin's is justly resorted to." Likewise of the end stages he stated: "The diagnosis cannot be made from such a gland or differentiated from the end-results of an acute lymphadenitis, tuberculosis, or syphilis."

Attention has been called to the case previously reported by Beaver and Johnson, in which the pathologic changes in the liver, spleen, and lymph nodes resembled very closely those found in the patient under discussion. In their patient, the clinical course of the disease was brief, but, in spite of this, sufficient portal obstruction had been produced to cause distention and ascites. Their search of the literature revealed two somewhat similar cases, that of Loygue and Clarion and that of Spangenberg. In each of these cases the general picture was that of a granulomatous process confined to the liver, spleen, and abdominal lymph nodes, and in each the histologic appearance of the tissues, while not typical, apparently resembled somewhat that of Hodgkin's disease.

There is a certain justification for temporarily including within the scope of the last named disorder other similar, although not identical, granulomatous diseases that are also of unknown etiology. At least it is on this premise that we must rely to support the diagnosis in this case. Others contend, perhaps more correctly, that so-called atypical cases of Hodgkin's disease should not be included in the same category, for such inclusion tends to impede discovery of etiologic factors. It must be admitted, however, that the criteria of histologic differentiation are not always well defined in this group of diseases and that there are multiple exceptions and overlappings, from varied granulomatous reactions to lymphosarcoma, leucemia, or pseudoleucemia. It is not entirely certain, moreover, that some of the conditions more recently described under the general heading "reticulosis" are not related to Hodgkin's disease. So long as indefinite definitions exist in such groups of diseases of unknown etiology, it will be extremely difficult to define sharply the scope of any one disease.

Ewing summed up this perplexing situation in his discussion of Hodgkin's disease with the following remarks: "Unfortunately, the clinical picture of Hodgkin's disease is not always associated with this particular histologic structure (proliferated endothelial cells, endothelial giant cells, plasma cells and eosinophilic leucocytes). . . Without the knowledge of the etiologic factor it remains almost as difficult as before to determine the true scope of this infectious granuloma, for all phases of the disease cannot be recognized with the same certainty. . . . In this dilemma one may follow the plan adopted by H. Ziegler, and include under Hodgkin's disease all conditions which seem to have any probable relation to the specific process. . . . There is a strong probability that the scope of the disease is very wide. . . ."

For these reasons and because of the clinical features of cervical adenopathy, which preceded the abdominal signs by a period of fourteen years, it seems advisable to record this unusual case as an example of atypical Hodgkin's disease. This is done with the full expectation that subsequent observations on similar cases may prove this classification to be in error. The possibility of a hitherto unrecognized disease entity, related to the infectious granulomatous processes, cannot be entirely excluded.

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ALLERGIC PHASES OF ARTHRITIS*

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SERUM sickness manifests itself in about 90 per cent of the cases where any foreign serum has been administered intravenously, and, according to various estimates, in from 8 to 80 per cent where the serum is given subcutaneously or intraspinally. The symptoms of serum sickness appear in from eight to twelve or more days following the injection of the serum, and are thought to be due to an excess of antibodies, thrown off into the blood and tissues, coming in contact with some of the remaining serum proteins. Definite joint symptoms occur in about 14 per cent of all cases of serum disease. Therefore, in every case of acute arthritis or arthralgia, inquiry should be made as to whether any foreign serum has recently been administered. If the joint involvement is an anaphylactic manifestation of serum sickness, it is usually accompanied by other symptoms, namely, fever, enlarged glands, and skin rashes which are usually of an urticarial nature, but may be erythematous, morbilliform, or scarlatiniform in type. The ideal treatment for serum sickness with its accompanying arthritis is epinephrine hypodermically, followed by ephedrine orally.

The joint manifestations of acute rheumatic fever are, in all probability, due to bacterial allergy. In this disease the symptoms migrate from one joint to another within the space of a few hours, and it does not seem possible that any localized infection could change its site so rapidly. The only logical explanation is that the joint involvement is due to a toxic or allergic effect from a focus of infection elsewhere in the body, usually the tonsils. The presence of infection alone is not sufficient to account for the rheumatic symptoms, because in subacute bacterial endocarditis the blood is filled with streptococci, but there is no arthritis; whereas in rheumatic fever, positive blood cultures are obtained with difficulty, yet many joints are affected.

The striking periodicity of the attacks of intermittent hydrarthrosis, as pointed out by Miller,[1] and the satisfactory response of this condition to injections of peptone, strongly suggest an allergic basis. Rowe[2] reported a case of intermittent hydrarthrosis that was proved to be due to food sensitization.

Chronic rheumatoid or atrophic arthritis affects young adults and middle-aged persons, mostly women. This form of arthritis is undoubtedly infectious in origin, inasmuch as brilliant cures have been reported from removal of foci of infection during the first year of the disease. Although cultural studies in rheumatoid arthritis frequently reveal streptococci, these are not always of the same type, as evidenced by their different effect on the various sugars, and by the failure of cross agglutination. Nevertheless, in spite of the difference in the causative organisms, the pathology of the joint lesions is always the same, sug-

*Received for publication, April 4, 1934.

Presented as part of a Symposium on Arthritis at a meeting of the George Washington University Medical Society, Washington, D. C., November 18, 1933.

gesting that the susceptibility or sensitization of the host is the important factor. A comparable situation exists in asthma and hay fever, where the same symptoms in different individuals may be due to sensitization to different and even unrelated substances.

Turnbull[3] stated that 60 per cent of a large series of arthritis patients, studied during a period of ten years, gave positive cutaneous reactions to tests with food allergens, and that elimination of the reacting foods from the diets of these patients resulted in a definite improvement in the arthritis. Rowe[2] also reports some cases of arthritis that are due to food sensitization, as evidenced by the prompt disappearance of the arthritis when certain foods were eliminated from the diet, and the recurrence of the trouble when the foods were again ingested. In Rowe's cases, however, skin tests were mostly negative and the causative foods were determined by means of his elimination diets.

The allergic hypothesis in arthritis obtains strong support from the experiments of Freiberg and Dorst,[4] in which they demonstrated that rabbits, after two or more injections of bacterial filtrates, developed characteristic joint lesions. These arthritic lesions became aggravated following each additional injection, and the rabbits gave positive skin reactions when tested intradermally with the bacterial filtrates.

Harkavy and Hebald[5] reported nine cases of asthma in adults who had also developed arthritis. In eight of these nine cases, the arthritis cleared up and the asthma greatly improved following the removal of the foci of infection. They concluded, therefore, that in these cases the arthritis, as well as the asthma, was due to bacterial allergy.

The possibility of food or bacterial allergy should be considered in every case of arthritis. A personal or family history of the common allergic diseases, namely, asthma, hay fever, urticaria, angioneurotic edema, eczema, or migraine headaches, greatly increases the likelihood that the arthritis also is due to some form of sensitization. Food sensitization should be ruled out by skin tests, both cutaneous and intradermal, with all the foods entering into the routine diet of the patient. If these food tests are negative, yet the history suggests food sensitization, then elimination diets are worthy of trial. Skin tests with stock polyvalent bacterial proteins should be done almost routinely in arthritis. Definite reactions with bacterial proteins are relatively rare, but when they do occur, treatment with corresponding vaccines, stock or autogenous, usually gives gratifying results. It must be remembered that although positive food tests usually give immediate urticarial reactions, bacterial reactions are almost always of the delayed inflammatory type.

In addition to the elimination of all foci of infection, cultures should be obtained from each of these foci for the preparation of autogenous vaccines and bacterial filtrates. A vaccine and filtrate are prepared from each different organism isolated from the cultures. These autogenous vaccines and filtrates are tested intradermally on the patient, and those that react are used for treatment. Treatments are given at weekly intervals, starting with a small dose, which is gradually increased according to the local reaction and the condition of the patient. Each dose should be regulated so as to produce a satisfactory

local reaction persisting on the arm for forty-eight hours, without any focal or constitutional symptoms. I have frequently seen an aggravation of the arthritis follow too rapid an increase in dosage. Crowe[6] of England, who devotes his entire time to the study of arthritis, claims that the right vaccine in the proper dosage is the ideal form of treatment for this disease. Vaughan[7] reports complete or partial relief from autogenous vaccine treatment alone, in 69 per cent of a series of one hundred cases of arthritis. Both Crowe and Vaughan stress the importance of small doses in the vaccine desensitization treatment of arthritis.

SUMMARY

Arthritis is frequently due to sensitization of the joint tissues, either to various foods, or to bacterial products liberated from foci of infection elsewhere in the body. The treatment consists of elimination of all offending foods, removal of all foci of infection, and specific bacterial desensitization.

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PHOSPHATASE IN HETEROTOPIC BONE FORMATION FOLLOWING TRANSPLANTATION OF BLADDER MUCOSA*

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Huggins[1] has shown that "bone forms around transplants of bladder, ureter, and renal pelvis epithelium to certain parietal fascias (the rectus sheath, fascia lata, subcutaneous tissue) and to muscle and synovial membrane in the dog." He reported that this is "true spongy bone with haversian canals containing fibrous and hemopoietic bone marrow." In a later study[2] he found that "in the presence of bone so formed the ratio of phosphatase activity to dry weight was very high, at least 2.5 to 8 times higher than the maximum, and from 10 to 20 times the mean values obtained for the control tissue without bone."

During the course of some other studies on bone phosphatase, we carried out an experiment similar to the phosphatase studies of Huggins, confining our studies to the bladder mucosa and the sheath of the rectus abdominus muscle using dogs as the experimental animals.

Procedure.—Under ether anesthesia and with aseptic precautions, a 2-inch incision was made in middle lower abdominal region, the urinary bladder exposed, an incision was made through the muscular coat down to the mucosa, the muscle was carefully pushed back until an area of the mucosa large enough to be grasped with the forceps was exposed, and the desired amount of mucosa was thus practically freed from muscle and excised. The incision in the bladder was closed with silk sutures. The sample of mucosa thus obtained was divided into two parts in such a manner that the exact weight of one would furnish a basis for the estimation of the weight of the other. In two instances both samples were weighed on a torsion balance, the "hook" of which had been sterilized with the operating instruments. One sample was sutured to the rectus sheath with silk and the abdominal incision closed with silk. The other sample was ground with (40 mesh) carborundum and extracted with water (containing $CHCl_3$) for twenty-four hours. It was then made to the smallest convenient volume with water and centrifuged. Phosphatase determinations were made on the supernatant according to the technic described by Jenner and Kay, 1932.[3] From the phosphatase activity of this sample that of the implant was calculated. These values are shown in Table I. After eleven to one hundred and eleven days the old incisions were reopened. In most instances cysts had formed and these were removed. In the first four cases these were cut in half, one portion being used for microscopic sections and the other for phosphatase determination. In

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Received for publication, April 19, 1934.

Part of the expense of this investigation was borne by a grant from the Division of Medical Science of the Rockefeller Foundation.

TABLE I

SYNTHESIS OR MOBILIZATION OF PHOSPHATASE DURING HETEROTOPIC OSSIFICATION RESULTING FROM TRANSPLANTATION OF BLADDER MUCOSA ON SHEATH OF RECTUS

(1)	(2)	(3)	(4)						
DOG	DAYS TRANSPLANT REMAINED IN DOG	WT. OF TRANSPLANT (APPROX.)	PHOSPHATASE IN TRANSPLANT	PHOSPHATASE IN REMOVED SAMPLE	(4)/(3)	P'ASE IN BLOOD PLASMA PER 100 C.C.	P'ASE IN CYSTIC FLUID	DEFINITE BONE FORMATION	INFECTION
		mg.	units	units		units	units		
1-98	22	130	0.134	0.164	1.2	10.9	–	No	Yes
2-85	20	67	0.042	1.500	35.7	7.5	–	Yes	Yes
3-42	49	195	0.265	2.380	9.0	3.6	–	Yes	No
4-2	11	145	0.164	0.194	1.2	11.6	–	No	No
5-23	111	180	0.092	2.400	13.0	–	–	Yes	No
6-70	27	588*	0.735	0 274	0.4	–	–	No	No
7-75	15	332*	0.170	1.13	6.7	–	–	Yes	No
8-7	56	777	2.0	2 3	1.2	–	–	Yes	No
9-6	56	825	1.60	1.9†	1.2†	–	1.9	Yes	No

*Exact weight.

†This bone was not used for phosphatase activity. These values were obtained from the cystic fluid alone.

the next four cases the whole cyst was ground in carborundum, extracted and made to volume as was the mucosa, and phosphatase determinations made on aliquots. In the last case, phosphatase determinations were made on samples of the cystic fluid only, the bone being cleaned and preserved. In this case the cystic fluid alone contained more phosphatase (phosphatase activity) than the original transplant.

When Dog 6 was reopened for removal of the transplant, it was found embedded in the rectus abdominal muscle entirely out of contact with the rectus sheath. No bone was found and the amount of phosphatase (phosphatase activity) found in it was less than when it was implanted in the dog twenty-seven days before.

Histologic sections were made from samples from the first four dogs only. In no case were complete serial sections made and thus it was not possible to ascertain beyond any doubt that no bone was formed in Dogs 1, 4 and 6, although on careful gross inspection no bone could be found.

The amount of bone formed does not seem to vary directly with the size of the sample of implanted bladder mucosa, although the largest implant made resulted in the largest bone (Dog 9-6). This may be due to the fact that the implants did not remain exactly in the original position, thus exposing varying areas to the rectus sheath. In any case the amount of the enzyme as measured by its activity is decidedly increased when bone appears. This is in agreement with the results of Huggins.[2]

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A NOTE ON THE MENSTRUAL INFLUENCE ON BLOOD MORPHOLOGY*

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IN THE course of a more extended investigation on various factors influencing blood morphology, a small group of consecutive analyzes were made to ascertain if there were a significant influence from menstruation on the blood picture. Four healthy athletic young women formed the nucleus of the group. To these were added 2 more equally athletic girls but with proved dysfunctional states of the pituitary associated with irregularity in the periods with shortened interval and normal or increased flow. Subject "W" had normal periods at from seven- to fourteen-day intervals; Subject "R" slightly more profuse periods at intervals of from eighteen to twenty-eight days. The essential basic data are given in Table I.

TABLE I

BASIC DATA

DATUM	NORMAL				PITUITARY	
	P.	D.	G.	K.	W.	R.
Age (yr.)	19	20	20	20	21	20
Height (cm.)	158.4	160.0	158.8	159.7	157.0	166.8
Weight (kg.)	55.0	60.5	56.4	58.2	53.2	66.8
Deviation (%)	−7.0	+1.0	±0.0	+3.0	−2.0	+8.0
Lung volume (%)	+7.0	+13.0	+7.0	+16.0	−2.0	−1.0
Total nitrogen (gm.)	7.1	9.1	7.5	12.5	5.6	10.3
Residual nitrogen (%)	8.4	7.4	9.1	6.7	7.2	8.7
Basal rate (%)	±0.0	−6.0	−10.0	−6.0	(−9.0)	−19.0
Sugar tolerance (%)	±0.0	±0.0	±0.0	±0.0	−75.0	−50.0

The data from the normal group attest the warrant of their designation. With Subject "W" we were unable to get a satisfactory basal rate, her true level falling materially below that recorded. Her presenting difficulty was promptly corrected by the therapeutic approach with pituitary medication. The individual menstrual habits are recorded in Table II.

TABLE II

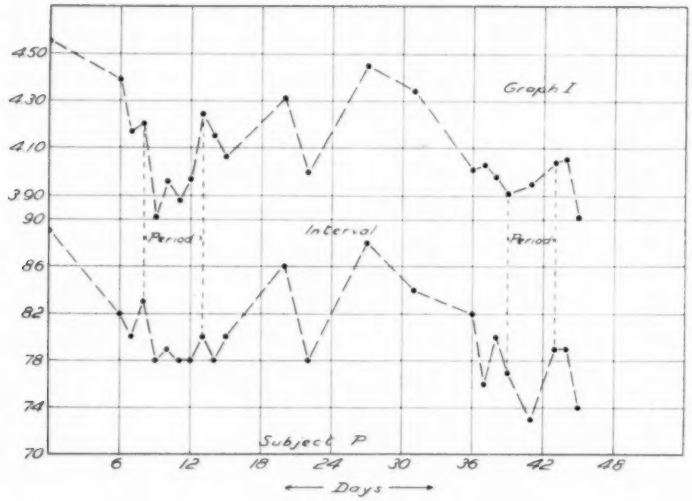
MENSTRUAL DATA

SUBJECT	ONSET	INTERVAL	PERIOD	NAPKINS
Normal				
P	13	28	7	5
D	13	25–30	3–4	3–4
G	12	28	4	3–4
K	13	28	4	3–4
Pituitary				
W	11	7–14	3–4	2–3
R	12	18–28	4	4–5

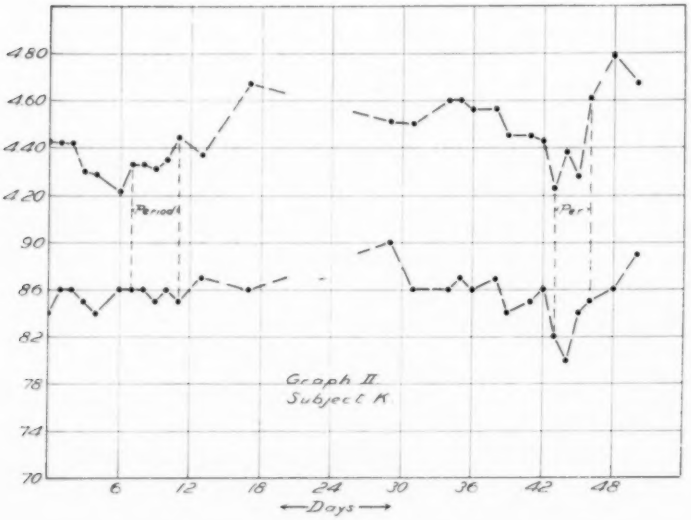
*From the Evans Memorial, Massachusetts Memorial Hospitals.
Received for publication, April 7, 1934.
Presented before the Biological Chemical Section of the American Chemical Society, Washington, March, 1933.

Subject "P" had long and ample periods; the others conform more nearly to the conventional mode.

All counts were made in the early morning with the subjects in a fasting state. So far as was possible, representative counts were made during the interval between periods together with daily records from two days before to



Graph I.

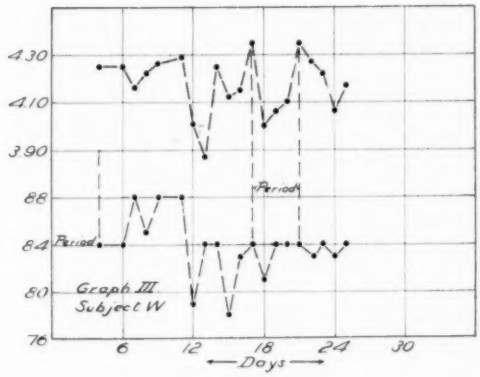


Graph II.

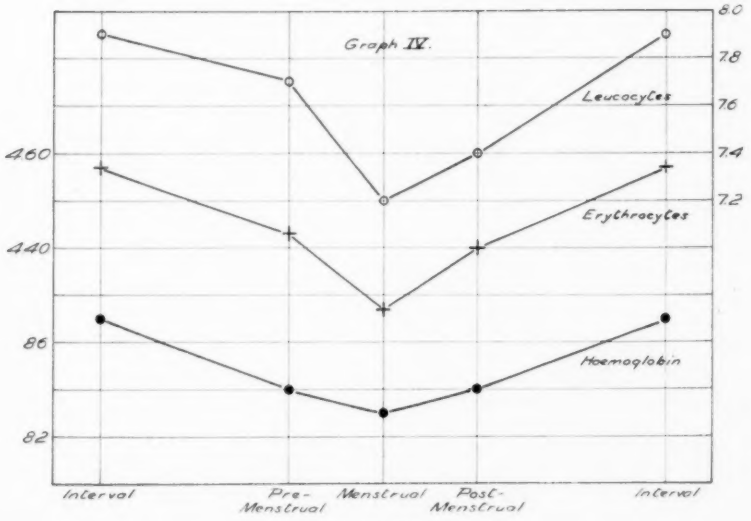
two days after the period. Bloods were taken before 8 P.M.; the hemoglobin estimated by the Dare and Newcomer procedures and red and white counts made in duplicate by the usual procedures using a standardized Levy (double Neubauer ruling) counting chamber. For the differential counts, duplicate smears were prepared with Wright's stain and 200 cells counted with each.

All of the curves show irregularities unassociated with any known influence. The general picture of the changes in hemoglobin and erythrocytes can best be shown graphically (Graph I).

This normal girl shows a definite drop of both components in the first period recorded; both the pre- and postmenstrual intervals reflect this depression. The second period is perhaps less well marked; transitory recovery of the erythrocyte



Graph III.



Graph IV.

count begins with the period. It will be noted that she was slightly delayed beyond her usual habitude.

Subject "K," whose record is given in Graph II, shows a variant of the preceding in that the first period demonstrates no change in hemoglobin and a slight rise in the red cell count during the first period. In the second, there is a gradual depression reaching culmination with inversion of the curve during the time of flowing. She, too, shows a protracted intermenstrual interval.

This is a partial record of the pituitary Subject "W" with marked fluctuations during the interval and but minor change in the hemoglobin during the

period. The erythrocytes demonstrate a sharp drop in the middle of the period with, however, the two highest and equal values recorded on the first and last days of the flow. The picture is one of a lack of normal control and may be felt to reflect the drain of her excessive and unregulated catamenia.

While the subjects were examined in a fasting state, it was impossible to arrange for basal examinations. We did not find, however, those high leucocyte counts following physical activity which have been frequently recorded in the literature. With but occasional minor exception, all of the values fell within the usual conventional normal range. The total leucocyte count showed much the same fluctuations and in perhaps a slightly more marked degree as were exhibited by the hemoglobin and red cells. The differential counts were seemingly unaffected by the catamenial influence. Taking the averages of all records for the four intervals determined by the presence or absence of menstruation, the results are given in Table III.

TABLE III

DIFFERENTIAL LEUCOCYTE COUNT

CELL	INTERVAL	PREMENSTRUAL	MENSTRUAL	POSTMENSTRUAL
Neutrophiles	52.0	50.0	50.0	51.0
Lymphocytes	39.0	38.0	39.0	38.0
Endothelial leucocytes	6.5	8.5	7.5	7.5
Eosinophiles	2.0	3.0	3.0	3.0
Basophiles	0.5	0.5	0.5	0.5

The lymphocytes are relatively slightly high, the neutrophiles correspondingly low. While the method of averages conceals individual variation, these figures represent the group and show no evidence of menstrual influence on the differential picture.

Still using average values, the general menstrual influence can be shown schematically.

All of the factors show a downward tendency during the flow with gradual recovery after its termination. The average change is not great, and the individual records of this small group would seem to indicate that none of them had a characteristic pattern. With Subject "W," the excessive blood loss seemed to be associated with some lack of control in the regeneration processes.

FACTORS INFLUENCING THE SEDIMENTATION RATE OF ERYTHROCYTES*

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FROM the time that Fåhraeus[1] in 1921 first elucidated his hypothesis "that the plasma contains a substance responsible for the increased sedimentation of erythrocytes" and "that the increased rate was due to their increased agglutination which in turn was dependent on the properties of the plasma" to the present day, many investigators have discussed this phenomenon in the literature. The variance of their findings and contentions encouraged me to attempt to evaluate the test by a combination of clinical and laboratory data. The data herein assembled are the result of nine years' observation of the sedimentation test done in an indiscriminate group of both hospitalized and ambulatory patients. The technic employed was the Friedlaender modification of the original Linzenmeier method.

To establish a normal basis for comparison, the sedimentation rate was performed on twelve healthy physicians and laboratory workers. The following tabulated results correspond with the normals given by Linzenmeier,[2] Flores,[3] Geppert,[4] Friedlaender,[5] and Baer and Reis.[6]

TABLE I

SEDIMENTATION TIME OF NORMAL INDIVIDUALS

	2 HOURS	3 HOURS	4 HOURS	5 HOURS	6 HOURS
6 men	1	2	1	1	1
6 women	1	1	1	1	2

Most observers agree that the chief influence on the sedimentation of erythrocytes is an inherent factor in the plasma, and even in the days of Galen,[7] when he described the crusta phlogistica, it was recognized that standing blood would settle in two layers, at different rates, as first noted by Hunter.[8] In 1897, Pfeiffer[9] studying on the formation of the "buffy coat" said, "an increase in the sedimentation of erythrocytes was due to an increase in agglutination, and that an increase in the fibrinogen factor might cause the corpuscles to congregate more rapidly and in this way further the agglutination." Dogiel,[10] Hayem,[11] and Valentin[12] all found that an increase in sedimentation was generally caused by increased agglutination. These facts suggested the channel of laboratory investigation of the plasma fraction, and a partition was therefore done.

To establish a group of controls, fractions were done upon five persons having a normal sedimentation rate.

*Received for publication, April 4, 1934.
Read before the New York Pathological Society, November 14, 1931.
This study was made possible by a grant from the Alice L. Filbert Fund.

The foregoing Tables III and IV, indicate that I have arbitrarily divided them into a "Fast Group" (Table III) ranging in time from five to sixty minutes and a "Slow Group" (Table IV) ranging from sixty to three hundred sixty minutes. In analyzing these tables very little alteration in the fractions is apparent in either the slow or fast groups, as compared with the controls or normals.

In the "fast group" there was an increase in the fibrin nitrogen, the average (Table V) being 0.18 as compared with the average normal of 0.06, and in the "slow group" it averaged 0.11. These results confirm the observations of Cordua and Hartmann[13] and Frisch and Starlinger.[14]

TABLE II

PARTITIONS UPON NORMAL INDIVIDUALS

PATIENT	TOT. N.	N. P. N.	PROT. N.	FIBRIN N.	EUGLB. N.	1 PSDO. GLN.	2 PSDO. GLN.	TOT. GLN.	ALB. N.	S. T.
1	1.16	0.02	1.14	0.07	0.15	0.19	0.15	0.49	0.58	5 hr. +
2	1.08	0.03	1.05	0.03	0.08	0.18	0.16	0.42	0.60	5 hr. +
3	1.10	0.04	1.06	0.01	0.19	0.20	0.08	0.47	0.58	3 hr. +
4	1.19	0.01	1.18	0.12	0.16	0.12	0.19	0.47	0.71	6 hr.
5	1.19	0.02	1.19	0.10	0.27	0.18	0.15	0.60	0.49	3 hr.

TABLE III

PARTITIONS UPON DISEASED INDIVIDUALS (FAST GROUP)

PATIENT	TOT. N.	N. P. N.	PROT. N.	FIBRIN N.	EUGLB. N.	1 PSDO. GLN.	2 PSDO. GLN.	TOT. GLN.	ALB. N.	S. T.
1	1.27	0.03	1.24	0.23	0.32	0.24	0.10	0.66	0.35	5 min.
2	1.49	0.30	1.19	0.15	0.27	0.29	0.00	0.55	0.49	5 min.
3	1.34	0.01	1.33	0.26	0.26	0.25	0.17	0.81	0.26	15 min.
4	1.11	0.03	1.08	0.21	0.27	0.24	0.07	0.58	0.29	15 min.
5	1.02	0.04	0.98	0.16	0.20	0.20	0.14	0.54	0.28	15 min.
6	1.28	0.22	1.16	0.13	0.18	0.19	0.13	0.50	0.43	15 min.
7	1.37	0.23	1.14	0.24	0.13	0.19	0.08	0.58	0.32	15 min.
8	1.38	0.27	1.11	0.15	0.23	0.17	0.16	0.56	0.40	15 min.
9	1.37	0.08	1.29	0.29	0.43	0.18	0.10	0.77	0.29	20 min.
10	1.02	0.02	1.00	0.18	0.29	0.06	0.11	0.14	0.36	25 min.
11	1.44	0.03	1.41	0.08	0.34	0.35	0.13	0.82	0.51	25 min.
12	1.35	0.03	1.32	0.33	0.25	0.28	0.10	0.63	0.36	40 min.
13	1.18	0.04	1.14	0.12	0.16	0.17	0.14	0.47	0.55	45 min.
14	1.04	0.02	1.02	0.11	0.18	0.18	0.11	0.47	0.44	50 min.
15	1.41	0.01	1.40	0.20	0.30	0.28	0.14	0.72	0.48	50 min.
16	1.13	0.01	1.12	0.13	0.15	0.18	0.19	0.52	0.47	60 min.

TABLE IV

PARTITIONS UPON DISEASED INDIVIDUALS (SLOW GROUP)

PATIENT	TOT. N.	N. P. N.	PROT. N.	FIBRIN N.	EUGLB. N.	1 PSDO. GLN.	2 PSDO. GLN.	TOT. GLN.	ALB. N.	S. T.
1	1.44	0.02	0.42	0.39	0.40	0.25	0.15	0.80	0.23	70 min.
2	1.13	0.08	1.05	0.13	0.11	0.22	0.11	0.44	0.48	85 min.
3	1.13	0.01	1.12	0.15	0.11	0.31	0.10	0.52	0.45	180 min.
4	1.04	0.03	1.01	0.01	0.05	0.24	0.08	0.37	0.63	180 min.
5	1.19	0.02	1.19	0.10	0.27	0.18	0.15	0.60	0.49	180 min.
6	1.10	0.04	1.06	0.01	0.19	0.20	0.08	0.47	0.58	180 min.
7	1.16	0.02	1.14	0.07	0.15	0.19	0.15	0.49	0.58	300 min.
8	1.18	0.03	1.05	0.03	0 08	0.18	0.16	0.42	0.60	300 min.
9	1.19	0.01	1.18	0.12	0.16	0.12	0.19	0.47	0.71	360 min.

The average euglobulin nitrogen was 0.17 in the normals, in the "fast group" 0.26, and in the "slow group" 0.12.

The average total globulin nitrogen in the normals was 0.49, 0.58 in the "fast group," and 0.15 in the "slow group." The increase in the globulin content in the fast sedimentation group parallels the results on Salomon.[15]

Table V shows that there is an appreciable increase in the fibrin nitrogen, euglobulin nitrogen, and the globulin nitrogen content in fast sedimentation cases, with only a slight increase in the slow cases, and a practically negligible increase in the pseudoglobulin 1 and 2 in both slow and fast.

TABLE V

AVERAGES OF TABLES II, III AND IV

	CONTROL	FAST	SLOW
Fibrin N.	0.06	0.18	0.11
Euglobulin N.	0.17	0.26	0.12
Pseudo Gl. 1 N.	0.17	0.21	0.21
Pseudo Gl. 2 N.	0.14	0.11	0.13
Globulin N.	0.49	0.58	0.51
Albumin N.	0.59	0.38	0.53

TABLE VI

THE EFFECT OF PROTEIN INGESTION UPON THE SEDIMENTATION TIME

	SED. TIME	N. P. N. MG./100	UREA N.	AMINO N.
Control	180	30.2	17.3	4.2
First hour	180	38.0	15.6	6.3
Second hour	180	37.3	16.8	8.4
Third hour	180	41.7	21.0	7.7
Control	50	24.6	10.3	5.6
First hour	35	26.5	10.5	4.9
Second hour	100	33.4	11.4	10.5
Third hour	45	39.0	12.8	4.9
Control	25	31.5	12.5	4.2
First hour	25	33.0	13.2	6.3
Second hour	20	33.0	14.0	7.0
Third hour	20	33.0	13.5	4.9
Control	30	23.9	12.8	7.0
First hour	20	25.2	11.3	7.7
Second hour	25	27.6	13.5	7.7
Third hour	25	29.3	13.2	9.1

The albumin nitrogen shows an average decrease in the fast cases with practically no change in the slow cases. This agrees with the findings of Mikulicz-Radecki.[16] This fall in the albumin nitrogen content in the fast cases can be accounted for by Nature's effort to compensate for the rise in fibrin nitrogen.

With this laboratory data in mind, an attempt was made to determine the effects of protein ingestion on the sedimentation rate.

A group of patients under study were each fed 150 gm. of lean meat and six ounces of water, and the sedimentation rate, nonprotein nitrogen, urea, and amino nitrogen, were determined each hour for the succeeding three hours. A control specimen was taken in each instance (Table VI).

In Case 1 (Table VI) all the fractions were progressively increased over the period of investigation with no change in the sedimentation rate. Cases 2,

3, and 4, which have been classified as "rapid," do not show any alteration in the sedimentation rate, in spite of the apparent increase in all fractions over the period. Therefore, it may be said that increasing the protein fractions does not alter either the slow or fast rates. Following up this trend of thought, the sedimentation rate of three cases of definite renal involvement was determined (Table VII).

TABLE VII

THE SEDIMENTATION RATE IN NEPHRITICS

1. Urine protein ++	Blood urea 22.7	N. P. N. 39.4		S. R. 180 min.
2. Urine protein +	Blood urea 30.0	N. P. N. 55.6		S. R. 51 min.
3. Urine protein ++++	Blood urea 124.5	Creatinine 21	Amno N. 49	S. R. 5 min.

After a review of all the findings regarding the nitrogen partition's influence on the sedimentation rate, it seems justifiable to state that in the face of such wide variations, no reliance can be placed on its influence on the sedimentation time.

To determine what influence glucose had on the sedimentation rate, a group of six individuals with normal blood sugars ranging from 0.078 to 0.142 mg./100 were selected for the glucose tolerance test. Each was given the standard amount of 1.74 gm. glucose per kilo of body weight, with sugar estimations and sedimentation rate readings at one- and two-hour intervals (Table VIII).

TABLE VIII

EFFECT OF GLUCOSE INGESTION UPON THE SEDIMENTATION RATE

PATIENT	CONTROL		FIRST HOUR		SECOND HOUR	
	S. R.	SUGAR PER CENT	S. R.	SUGAR PER CENT	S. R.	SUGAR PER CENT
1	20	0.113	20	0.187	20	0.182
2	20	0.109	20	0.195	20	0.175
3	45	0.142	45	0.258	45	0.340
4	120	0.097	155	0.117	150	0.103
5	120	0.078	30	0.142	85	0.138
6	155	0.095	85	0.158	110	0.110

It will be observed that in the fast cases the sedimentation rate remained constant despite an increased sugar percentage, whereas in the slow group it showed a decrease in the rate along with an increase in the sugar percentage. In an attempt to correlate these findings, a group of recognized diabetic patients were studied (Table IX).

TABLE IX

SEDIMENTATION RATE IN DIABETIC PATIENTS

	PATIENT	BLOOD SUGAR	SEDIMENTATION RATE
1	G.	0.445	33 min.
2	S.	0.156	51 min.
3	G.	0.297	23 min.
4	A.	0.240	108 min.
5	S.	0.156	382 min.
6	N.	0.193	205 min.
7	N.	0.096	357 min.

In the glucose tolerance control patients, it is apparent that in Cases 4, 5, and 6, or fast sedimentation rate, the higher the sugar percentage, the slower the rate; this seems to be the reverse in cases of clinical hyperglycemia.

The effect of the ingestion of food on the stability of the blood was shown by Rourke and Plass[17] to be negligible and confirms De Courcy's[18] observations.

Although the blood plasma has been credited with containing the necessary "something" which influences the sedimentation rate of the erythrocytes, it is certain that the cells themselves play a most important part. Groedel and Hubert[19] state that the erythrocytes are the most important single blood component in the sedimentation rate. In cases of high hemoglobin and a normal red blood cell count the rate is usually normal or slightly slowed. They also noticed that an increased rate is more common in blood with low hemoglobin, differing from Westergren,[20] who claimed that the sedimentation rate is influenced chiefly by the hemoglobin content. Rubin's and Smith's[21] findings in this relation were in accord with those of Groedel and Hubert.

Tables X and XI refer to a study of eighteen patients upon whom red blood cell count, hemoglobin, and sedimentation time were taken. They were divided

TABLE X

RELATION OF RED CELL COUNT, HEMOGLOBIN AND SEDIMENTATION TIME (FAST GROUP)

NAME	R. B. C.	HEMOGLOBIN	SEDIMENTATION TIME
1	1,950,000	40%	1 min.
2	3,240,000	54%	5 min.
3	3,300,000	60%	13 min.
4	3,680,000	86%	15 min.
5	1,790,000	32%	15 min.
6	2,240,000	50%	15 min.
7	2,800,000	40%	15 min.
8	3,520,000	64%	20 min.
9	2,230,000	50%	20 min.
10	3,560,000	67%	25 min.
11	3,670,000	70%	30 min.
12	3,330,000	70%	30 min.
13	4,220,000	67%	45 min.

TABLE XI

RELATION OF RED CELL COUNT, HEMOGLOBIN AND SEDIMENTATION TIME (SLOW GROUP)

NAME	R. B. C.	HEMOGLOBIN	SEDIMENTATION TIME
1	4,600,000	84%	60 min.
2	3,700,000	58%	70 min.
3	2,940,000	50%	70 min.
4	2,200,000	32%	85 min.
5	4,725,000	74%	100 min.

into the "fast" and "slow" groups according to the sedimentation time. As can be seen in the "fast" group, where all the rates of blood were below sixty minutes, when there was a low red cell count and hemoglobin, a fast sedimentating blood was usually present. However, a sufficient number of exceptions occur to prevent definite conclusions being drawn.

Table X shows hemoglobin varying from 32 to 86 per cent and red blood cells 1,790,000 up to 4,220,000 with the sedimentation rates varying from one minute to forty-five minutes.

Table XI shows the hemoglobin from 32 to 84 per cent and the red blood cells from 2,200,000 to 4,600,000, and the sedimentation rate from sixty to one hundred minutes.

There does not seem to be any consistency in relationship or parallelism between the hemoglobin, red blood cells and sedimentation time in either group. This can be seen by comparing Case 10 of the "fast" group (with hemoglobin 32 per cent, red blood cells 1,790,000 and sedimentation time of fifteen minutes) with Case 17 of the "slow" group (with hemoglobin 32 per cent, red blood cells 2,200,000 and a sedimentation time of eighty-five minutes); and again, Case 2 in the "fast" group (hemoglobin 86 per cent, red blood cells 3,680,000, sedimentation time fifteen minutes), with Case 15 in the "slow" group (84 per cent hemoglobin, red blood cells 4,600,000, and sedimentation time of sixty minutes). This discrepancy is noted throughout. Variations occurred sufficiently often in this series of pathologic states to prevent establishing a definite ratio between the red blood cells, hemoglobin, and sedimentation time. These findings agree with the observation of Hunt,[22] who claims that in normal bloods the red blood cells, hemoglobin, and sedimentation rate show a direct relationship, and also believes that as the blood count decreases the sedimentation rate increases, but no definite parallelism exists.

It is an established fact that the percentage of fibrin of the blood in simple anemia, aplastic anemias, and polycythemia is found almost consistently within normal limits. In the leucemias and pseudoleucemias there is only slight, if any, increase. In the primary anemias there is a diminished fibrin. Gram[23] found an increase sedimentation of erythrocytes in anemias that closely paralleled the decrease in the red blood cells, and that the fibrin values did not increase unless there was secondary infection.

Any marked variation of the cell count, either above or below normal limits, affects the sedimentation rate. This was demonstrated in two cases of polycythemia (Table XII) and five cases of secondary anemia (Table XIII) (with established causes) that were studied.

TABLE XII

SEDIMENTATION TIME IN TWO CASES OF POLYCYTHEMIA

NAME	R.B.C.	HEMOGLOBIN	S. T.	R.B.C. VOL.	PLASMA
1	8,200,000	132%	18 hours	71%	29%
2	8,150,000	110%	5 hours	72%	28%

TABLE XIII

SEDIMENTATION TIME IN FIVE CASES OF SECONDARY ANEMIA

PATIENT	R.B.C.	HEMOGLOBIN	SEDIMENTATION TIME
1	3,240,000	54%	5 min.
2	2,350.000	55%	6 min.
3	2,350,000	32%	23 min.
4	2,240,000	50%	22 min.
5	3,590,000	58%	43 min.

The sedimentation rate in relationship to cell volume and total solids was next studied (Table XIV).

TABLE XIV

RELATION TO THE SEDIMENTATION TIME TO CELL VOLUME AND TOTAL SOLIDS

PATIENT	SEDIMENTATION TIME	R.B.C. VOL. PER CENT	TOTAL SOLIDS PER CENT
1	5 min.	20	16.0
2	15 min.	29	18.0
3	25 min.	36	22.3
4	300 min.	45	22.8
5	18 hours	71	21.0
6	5 hours	72	28.0

"In blood the sedimentation rate of the erythrocytes is, roughly, inversely proportional to the cell volume, provided other factors are unchanged." This was stated by Rourke and Plass[17] and emphasized by additional workers (De Courey[18] Gram,[23] and Rubin and Smith[21]). Rourke and Plass, in their experimental work on the effect of blood dilution on the sedimentation rate, concluded that it is very important that the result of a sedimentation test should not be interpreted in terms of infection or of other tissue damage, without consideration being paid to the concentration of the blood (cell volume).

A study of the red blood cells and sedimentation rate led to the investigation of the relationship which exists between the leucocytes and sedimentation rate (Table XV). No particular class of pathologic conditions was selected; hospital cases were taken at random.

TABLE XV

RELATION OF THE SEDIMENTATION TIME TO LEUCOCYTE COUNT (FAST GROUP)

PATIENT	SEDIMENTATION RATE	W.B.C.
1	5	10,000
2	5	10,200
3	5	21,300
4	5	25,250
5	5	12,500
6	5	17,000
7	10	7,000
8	10	19,950
9	15	10,800
10	15	9,800
11	15	22,000
12	15	11,700
13	15	11,400
14	15	14,000
15	15	9,800
16	15	13,000
17	15	12,400
18	15	10,200
19	15	33,550
20	15	25,600
21	15	17,500
22	20	11,400
23	20	20,000
24	20	11,200
25	25	10,000
26	25	27,000
27	25	8,000
28	25	25,200
29	30	8,800
30	35	9,250
31	50	8,000

According to Halliburton,[24] Lackschewitz,[25] Berggrün,[26] and Dochez[27] many infections and diseases such as pneumonia, tuberculosis, pleurisy, peritonitis, acute rheumatic fever, erysipelas, and scarlatina show an increase of fibrin in the blood. Pfeiffer[9] in reporting his extensive investigations of the fibrin content of the blood in disease, stated that in general the fibrin was increased. He also noted that within the increased fibrin there was a leucocytosis, and with a decrease in the leucocytes a normal fibrin content existed.

The high leucocyte counts shown in Tables XV and XVI were from specimens taken from a diversified group of acutely ill hospital patients suffering from meningitis, pleurisy, abscesses in the lungs and peritoneal cavity, pneumonia, erysipelas, peritonitis, and salpingitis. They verify the reports of other investigators that leucocytosis by its increase in fibrin content increases sedimentation rate.

TABLE XVI

RELATION OF SEDIMENTATION RATE TO THE LEUCOCYTE COUNT (SLOW GROUP)

PATIENT	SEDIMENTATION RATE	W.B.C.
1	70	5,900
2	70	11,200
3	85	7,800
4	85	9,600
5	115	11,160
6	115	9,850
7	120	10,300

TABLE XVI

FAST GROUP

PATIENT	SEDIMENTATION RATE BEFORE	SEDIMENTATION RATE AFTER WASHING IN NORMAL SALINE AND REPLACING IN OWN PLASMA
1	45 min.	35 min.
2	35 min.	35 min.
3	20 min.	25 min.
4	15 min.	15 min.
5	25 min.	40 min.
6	10 min.	10 min.
7	35 min.	23 min.

It was thought that some information of value might be obtained by crossing the elements of fast sedimentating blood with the elements of slow sedimentating blood, using the same blood groups. Hunt observed that by exchanging so-called slow cells with fast plasma he obtained practically the same rate of the cells. If the cells of this blood were transferred to the slow plasma, the results were almost the same as when the original cells were in the plasma. He said, "as this phenomena occurred quite consistently in all samples of blood obtained from patients who were in compatible groups, it would seem that the substance controlling the sedimentation of the erythrocytes is contained in the plasma."

Rourke and Plass[17] have shown that when centrifuging for as long as twenty minutes at 2,500 r.p.m. the stability of the remixed blood is not different from that of the freshly drawn sample.

In order to determine what effect washing the cells in normal saline solution and replacing them in their own and other sera would have, they were first centrifuged and then replaced in their own plasma. The results were practically the same as the original sedimentation rate. The erythrocytes were then centrifuged out of their plasma, washed in normal saline, and replaced in their own plasma. Tables XVI and XVII show the results.

TABLE XVII

SLOW GROUP

PATIENT	SEDIMENTATION RATE BEFORE	SEDIMENTATION RATE AFTER WASHING IN NORMAL SALINE AND REPLACING IN OWN PLASMA
1	240 min.	180 min.
2	240 min.	420 min.
3	180 min.	180 min.
4	180 min.	180 min.
5	180 min.	210 min.

In twelve cases there was only one blood in which the rate was appreciably decreased (Case 2 of the slow group) and one in which the rate was increased (Case 5 of the fast group). The conclusions from these findings can logically be that changes in the physical environment of the erythrocytes have very little influence upon the sedimentation rate, and can be practically ignored when cross sedimentation tests were made.

The "fast red cells" after centrifuging and washing with normal saline were next crossed with "slow plasma." The result of seventeen such experiments is shown in Table XVIII.

TABLE XVIII

"FAST" RED BLOOD CELLS CROSSED WITH "SLOW" PLASMA

R.B.C.	PLASMA	CROSSED	PER CENT CHANGE IN TIME
5	120	5	0
10	240	300	+2900
10	85	20	+ 100
15	120	50	+ 233
15	120	30	+ 100
15	300	120	+ 700
15	180	180	+1100
15	240	420	+ 833
20	140	35	+ 75
20	180	240	+1100
25	360	360	+1340
25	180	300	+1100
30	240	240	+ 700
30	180	45	+ 50
35	180	360	+ 928
45	240	420	+ 833
45	120	55	+ 22

A tremendous change varying from 22 per cent to 2,900 per cent in slowing of the rate is noted here (Table XVIII).

This experiment was then repeated, using the reverse process of mixing slow red cells with fast plasma. The result in fourteen cases is shown in Table XIX.

TABLE XIX

"SLOW" RED BLOOD CELLS CROSSED WITH "FAST" PLASMA

R.B.C.	PLASMA	CROSSED	PER CENT CHANGE IN TIME
85	10	35	–59
120	15	30	–75
120	20	55	–54
120	5	20	–83
120	15	30	–75
140	20	50	–64
180	35	35	–80
180	20	25	–80
180	25	10	–94
240	45	70	– 8
240	30	95	–60
240	45	70	– 8
300	15	15	–95
360	25	60	–85

Here there is a marked acceleration of the rate, varying from 8 per cent to 95 per cent. In reviewing both tables, the findings are in accord with those of other workers. The plasma is the influencing factor and is more pronounced when fast cells are mixed with slow plasma than the reverse, although in the latter instance also, the controlling influence of the plasma is quite marked.

The surface tension in bloods of varying sedimentation rate was next determined. These were tested by the Du Noy Tensionmeter, and the results tabulated in fast and slow groups (Tables XX and XXI).

The average sedimentation rate was twenty-one minutes and the average surface tension was thirty dynes (Table XX).

TABLE XX

FAST SEDIMENTATION RATE

PATIENT	SEDIMENTATION RATE	SURFACE TENSION M.M.
1	10	38
2	10	36
3	15	34
4	20	32
5	25	30
6	30	30
7	35	23

TABLE XXI

SLOW SEDIMENTATION RATE

PATIENT	SEDIMENTATION RATE	SURFACE TENSION M.M.
1	85	12
2	140	2
3	180	2
4	180	4
5	180	3
6	195	5

The average sedimentation rate was one hundred sixty minutes and the average surface tension was four and six-tenths dynes (Table XXI).

These findings suggest that the faster the sedimentation rate, the greater the surface tension. Since surface tension is due to the fact that the molecules

within a liquid are subjected to equal forces of attraction on all sides, at the surface these forces act only on one side of the molecule and thus tend to pull them inward. This causes the surface to pull itself together so as to occupy the least possible area, and it is this force which constitutes surface tension. Thus it would seem that with an increased molecular concentration in fast sedimentation rate blood, the greater the tension, and vice versa.

Surface tension was then checked upon bloods which had been crossed and the results are shown in Tables XXII and XXIII.

Average slow plasma, 180; average red blood cells, 20; average surface tension, 4.7 m.m.

TABLE XXII

SURFACE TENSION FOLLOWING CROSSING "SLOW" PLASMA WITH "FAST" RED CELLS

PATIENT	SLOW PLASMA	FAST RED BLOOD CELLS	SURFACE TENSION M.M.
1	85	10	15
2	180	20	1
3	180	35	1
4	180	15	6
5	180	10	3
6	195	25	1
7	240	30	6

TABLE XXIII

SURFACE TENSION FOLLOWING CROSSING "FAST" PLASMA WITH "SLOW" RED CELLS

PATIENT	FAST PLASMA	SLOW RED BLOOD CELLS	SURFACE TENSION M.M.
1	10	180	24
2	10	85	24
3	15	180	24
4	25	180	26
5	25	195	30
6	30	240	21
7	35	180	26

Average fast plasma, 21; average slow red blood cells, 180; average surface tension, 25 m.m. (Table XXIII).

From these crossed sedimentation and surface tension readings it seems that where slow plasma and fast cells have been mixed, the surface tension about equals that of the slow sedimentating blood; whereas a mixture of fast plasma and slow cells results in a lowering of the surface tension.

A group of thirty postoperative patients were carefully studied. They were all clean cases, free from pre- and postoperative infections. The sedimentation rate was taken preoperatively, six hours postoperatively, and on the fifth and ninth postoperative days. The chemical blood and urine, as well as a red blood cell and white blood cell count, were simultaneously studied. All of these postoperative cases showed an acceleration of the sedimentation rate and were arbitrarily classed as "fast," some being as rapid as ten minutes and all under sixty minutes. This would seem to coincide with the findings of others, in that it indicates a disturbance of the balance, by autolysis of tissue secondary to surgical trauma.

Greisheimer[25] has shown that the sedimentation rate varies from day to day in healthy individuals, but always within normal limits. This he attributes to the incidental wear and tear of life on body tissue.

In the absence of pyrexia after the initial postoperative rise of temperature subsiding by the fifth day, and the normal leucocyte count, I think we are justified in assuming that the increase in rate is due to a disturbed balance caused by the absorption of tissue destruction, or as has been suggested, that the presence of proteolytic ferments at the site of operation may disturb the fine chemical balance necessary to maintain the equilibrium between the cells and plasma.

Among the gynecologic conditions most often affecting the sedimentation time, pelvic infections, especially the acute inflammations, are most prominent. In the pelvis as in other anatomical sites where infection exists, an increase in sedimentation rate takes place. Many gynecologists have placed great faith in this test as a diagnostic and prognostic aid and have laid considerable stress upon it as a guide as to when to operate for the relief of pelvic infections.

In a former article (Cherry[29]) in which the results of the sedimentation time in pelvic infections were evaluated, it was shown that such an index could not be followed, and that the clinical picture, leucocyte count, and elevation of temperature were more dependable guides.

Benign, uncomplicated pelvic neoplasms as a rule do not show any decided change from normal in the sedimentation rate. However, in the presence of necrobiosis and infection there is an acceleration of the sedimenting velocity, probably due to increase in blood fibrin and decrease in erythrocytes. Benign ovarian neoplasms and fibromyomas showed a normal sedimentation rate in a group of thirty cases. In the presence of secondary anemia, where uterine bleeding is a pronounced symptom, acceleration occurs. These observations agree with those of other workers in this field.

The sedimentation time as a rule is accelerated in all types of malignant disease, irrespective of its location. The acceleration becomes markedly increased in the presence of metastases, necrosis, infections, and anemia. Rubin[30] believes that an increasing sedimentation rate is an indication of the advancement of the disease. This increase in the sedimentation time in cases of cancer can be especially applied to those instances in which infection has not involved either the primary or metastatic growths. My own observations confirm these findings.

All observers agree that during pregnancy erythrocytic sedimentation shows an acceleration after the fourth month of gestation, and is continued into the puerperal state, returning to normal at its termination. Fåhraeus considered the acceleration of great value in the diagnosis of early pregnancy. This view is not confirmed by later observers. Hirst and Long,[31] Baer and Reis, Falta[32] all agree that as a diagnostic aid in pregnancy it has no value. There is no doubt that the pregnant state brings about definite changes in the maternal blood, and with these alterations the sedimentation time of red cells is changed. Sakae and Tsutsumi[33] believe that the ratio of the blood volume to the erythrocytes is greater and that the corpuscles agglutinate more rapidly because of

the increase of the globulin and fibrinogen, or because of a decrease in the albumin contents. Gram considered that the lowered percentage of cell volume in the early months of pregnancy and the increase in fibrin in the later months caused the acceleration. It seems that there are several factors influencing this increase of sedimentation time. As pointed out by Bland[34] the anemia accompanying pregnancy would show a lowered cell volume, thus increasing the sedimentation time. He furthermore indicated that as a leucocytosis attends the later months of pregnancy, there is an increase in the fibrin content of the blood which has been believed to hasten the sedimentation velocity.

During the puerperium the sedimentation rate is rapid for the first ten or twelve days, with a gradual decline to normal upon the termination of the involuting process. This process can be likened to the one following operative procedures, where the sedimentation time is always rapid.

SUMMARY

1. Plasma partition showed increase in the fibrin nitrogen, euglobulin nitrogen, and globulin nitrogen in both slow and fast groups of sedimentating bloods, but was more marked in the fast group.

2. A fall in the albumin nitrogen in the fast group is at the expense of the rise in fibrin and euglobulin nitrogen.

3. No relationship exists between renal involvement and the sedimentation rate.

4. The glucose tolerance test shows a decreased rate with an increase in the blood sugar percentage in controls; the reverse seems to be found in diabetic patients.

5. Normal red blood counts and hemoglobin show a fairly constant ratio with the sedimentation time.

6. Variations of the red blood count from normal affect the sedimentation rate.

7. Leucocytosis increases sedimentation rate, but this is due to the increased fibrinogen content in the blood. Leucopenia gives a normal percentage.

8. Crossed sedimentation adds to the evidence that the plasma is the controlling factor on the sedimentation rate.

CONCLUSIONS

It would seem from the foregoing review of the literature, clinical data, and laboratory findings that changes in the blood sedimentation time are caused by disturbing the fine balance between the cellular and fluid elements in the circulation.

Two factors apparently influence the change of rate, cell volume and the variations of fibrin, euglobulin, and globulin in the plasma. It has been shown that these variations from normal occur in so many pathologic states, thus influencing the settling of the erythrocytes, that it is impossible to affix definitely a standard sedimentation rate to any disease.

To rely upon this test alone, without other laboratory data, is unwise. To interpret this test for diagnostic purposes, the cell volume and nitrogen partitions must be determined if it is to be of any value.

From a clinical point of view, the sedimentation test alone is confusing, because individuals react biochemically to disease in different ways, thereby producing variations in rate in the same pathologic state. This has been observed many times; of two persons having the same disease, one will frequently show a sedimentation rate in the fast group and the other in the slow group.

Occasionally it is of aid in obscure cases by suggesting a lead to follow in a new line of investigation.

As a prognostic aid it is of slight help. A very rapid sedimentation rate does not by any means indicate a poor outlook for recovery; it indicates that toxic absorption and tissue destruction are progressing so rapidly that production of biochemical changes in the blood stream is taking place, and this causes the imbalance of the sanguineous elements.

The leucocyte count or filament-nonfilament study in conjunction with the clinical picture is of much greater aid than the sedimentation rate.

Thanks are due Dr. John A. Killian, Director of the Department of Biochemistry, and Dr. J. Carlind Browne, of the Medical Department, of the New York Post-Graduate Medical School and Hospital, for their kind cooperation.

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LIPASE AND ESTERASE IN THE BLOOD SERUM*

Their Diagnostic Value in Pancreatic Disease

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WITH the hope of finding a procedure of greater value than the existing tests in the diagnosis of pancreatic disease, an investigation of the lipase activity of the blood serum was begun one and a half years ago. Rona and Pavlovic, using a stalagmometric method with tributyrin as a substrate, found that serum lipase is inactivated by both quinine and atoxyl, whereas atoxyl inactivates hepatic lipase, and quinine inactivates pancreatic lipase. Methods based on this work had been promising, but had not given uniform results in the hands of various workers and were discarded in favor of one based on the experiments of Cherry and Crandall. These authors demonstrated that an enzyme capable of hydrolyzing olive oil, normally not present, appeared in the blood stream following ligation of the pancreatic ducts of dogs, and that the activity of the enzyme, esterase, normally present in the blood, as measured by hydrolysis of ethyl butyrate or tributyrin, did not show uniform increases. This demonstration indicated that the appearance of an enzyme capable of splitting olive

*From the Mayo Clinic.
Submitted for publication, May 5, 1934.

oil in the serum was specific for pancreatic injury, and seemed to offer more logical grounds for the development of a simple, quantitative test.

With these data in mind, the serum of 162 patients has been examined to date, to determine if enzymes capable of splitting olive oil and simple esters are normally present in the serum of man, if the increase of serum lipase activity is specific for pancreatic injury, and if the determination of their activity may be used clinically in the diagnosis of pancreatic disease. Our results encouraged the offering of this preliminary report.

METHODS

The serum was obtained from a fasting patient. A modification of the Loevenhart method, used by Cherry and Crandall, with a slightly different buffer solution, was employed. To 1 c.c. of serum were added 3 c.c. of distilled water, 1 c.c. of absolute ethyl butyrate, and 0.5 c.c. of Sorensen's phosphate buffer adjusted to the pH of 7. After shaking thoroughly, the mixture was allowed to stand in the incubator at 40° C. for twenty-four hours, and then was titrated with twentieth normal sodium hydroxide, using 3 drops of 1 per cent phenolphthalein as an indicator, and bringing the solution to the faintest permanent pink. The same method was used in testing the action of lipase, except that 2 c.c. of a 50 per cent emulsion of olive oil in water, emulsified with 5 per cent acacia (prepared by the Abbott Laboratories) was used, and 0.3 c.c. of 95 per cent alcohol was added to each tube before titrating.

RESULTS

Esterase.—An enzyme capable of splitting simple esters (esterase) was present constantly in the serum of 162 patients. The fluctuations in its activity bore no constant relation to the condition of the pancreas, and were never marked.

Lipase.—An enzyme capable of splitting olive oil (lipase) was present in the serum of 158 of the 162 patients.

The values for lipase activity of patients without known pancreatic disease and of others with known pancreatic disease were studied. In 130 patients without known disease of the pancreas, the range of lipase varied between 0 and 6.4 c.c. twentieth normal sodium hydroxide (per 1 c.c. of serum). In 119 patients, or 92 per cent of the 130, these lipase values fell below 2 c.c., and in 11, or 8 per cent, the values were 2 c.c. or higher. On the other hand, in 32 patients with pancreatic disease, demonstrated at operation or at necropsy, the range of serum lipase activity varied between 0.4 and 10.2 c.c. of twentieth normal sodium hydroxide (per 1 c.c. of serum). In 19, or only 59 per cent, was the lipase activity less than 2 c.c., and in 13, or 41 per cent, the value was 2 c.c. or more (Table I).

The contrast in percentages indicates a relationship between pancreatic disease and serum lipase activity higher than 2 c.c. (for each 1 c.c. of serum).

If the 24 cases in which there was a lipase activity of 2 c.c. or more, in terms of twentieth normal sodium hydroxide (for 1 c.c. of serum), be critically examined for evidence of pancreatitis, activity of such values appears significant

TABLE I

SUMMARY OF MATERIAL

DIAGNOSIS	LIPASE IN TERMS OF C.C. N/20 NaOH PER 1 C.C. OF SERUM				CASES
	1.9 C.C. OR LESS		2 C.C. OR MORE		
	CASES	PER CENT	CASES	PER CENT	
No organic disease of the liver, biliary tract, or pancreas	54	95	3	5	57
Hepatic disease Portal cirrhosis (7 cases) Toxic cirrhosis (8 cases) Metastatic carcinoma of liver (4 cases)	18	95	1	5	19
Traumatic or malignant stricture of the common bile duct	13	95	1	7	14
Cholecystic disease	7	100	0	0	7
Miscellaneous	6	86	1	14	7
Disease of the common bile duct (without operative discovery of pancreatic disease)	21	81	5	19	26
Total without known pancreatic disease	119	92	11	8	130
Pancreatitis, operative finding	11	55	9	45	20
Carcinoma of head of pancreas	8	66	4	33	12
Total with known pancreatic disease	19	59	13	41	32
Total	138	85	24	15	162

in the diagnosis of pancreatic disease (Table I). Pancreatitis was present in 9 cases, carcinoma of the head of the pancreas in 3 cases, and carcinoma of the ampulla of Vater in one case. In other words, in 13 or 54 per cent of those cases with lipase activity higher than 2 c.c. (for 1 c.c. of serum), pancreatic disease was known to be present (Table I). The explanation of the increased lipase values in the remaining 11 cases (if they are increased and not high normal values) must be sought in disease of the pancreas, intestinal mucosa, liver, or spleen, since Cherry and Crandall have shown that an enzyme capable of splitting olive oil is present in appreciable amounts only in the tissues of these organs. Splenic disease does not occur in these cases. In two cases there was injury caused by obstructive jaundice from carcinoma of the gallbladder, and of toxic hepatitis associated with lobar pneumonia. Hepatic injury might be advanced in explanation of the lipase values. However, this is unlikely, since in thirty-eight other patients with hepatic injury, in many instances much more severe, due to portal and toxic cirrhosis, metastatic carcinoma, obstructive jaundice secondary to benign traumatic stricture, and carcinoma of the gallbladder and ducts, the lipase value was below 2 c.c. (Table I). In fact, it seems as reasonable to suppose that the late postoperative rise in serum lipase values of the patient with carcinoma of the gallbladder was due to pancreatitis which had followed introduction of infection into the biliary passages, and that the high lipase values of the patient with lobar pneumonia and toxic hepatitis were due to associated toxic pancreatitis. In the five cases of stone in the common bile duct in which there were symptoms of cholangeitis, pancreatitis is a more reasonable explanation of the elevated serum lipase content than any possible hepatic injury. In the remaining cases (duodenitis, inoperable carcinoma of the stomach, malfunctioning gastroenteric stoma, and peritonitis) although

hepatic injury is hardly a probability, some destruction of intestinal mucosa may have taken place. Although these data do not prove conclusively that an elevated serum lipase value is specific for pancreatic disease, they do show that pancreatic disease accounts for the rise to more than 2 c.c. in serum lipase values in more than 50 per cent of cases in which the value is thus elevated and probably for a much larger percentage. If combined with symptoms of biliary stone or infection, or suggestive of pancreatitis, the finding of lipase activity higher than 2 c.c. was strong confirmatory evidence of pancreatic disease.

If the twelve cases in which the lipase value was 3 c.c. or more are studied in the same way, nine patients, or 75 per cent, are known to have pancreatitis and three, or 25 per cent, may be assumed to have pancreatitis. The reasons for postulating pancreatitis in the cases of carcinoma of the gallbladder, toxic hepatitis due to pneumonia, and peritonitis as the cause of the lipase activity, have been given. In other words, pancreatitis is the most likely explanation in all twelve cases in which the lipase values are high. On further study, a reading of 3 c.c. or more may prove to be almost certain evidence of pancreatic disease.

Carcinoma of the ampulla of Vater and of the head of the pancreas, and acute pancreatitis, are the two diseases of the pancreas so far associated with increased activity of serum lipase. The increase seems to follow both inflammation of the gland and obstruction of the ducts. In 43 per cent of the cases of carcinoma of the head of the pancreas in which examinations have been made so far, the serum lipase readings were higher than 2 c.c. Such a percentage may be greater than might be expected theoretically. Baldwin has shown that the main and accessory pancreatic ducts anastomose at about the level of the neck of the gland, and that both empty separately into the duodenum in more than 80 per cent of cases. When the ducts are so arranged, the tumor, if in the head of the gland, must occlude both ducts in order that obstruction may occur. Tumors of the neck or body of the gland may produce obstruction by occluding the main pancreatic duct before the accessory and main pancreatic ducts anastomose. When the duodenal opening of the accessory pancreatic duct is not patent, involvement of the main duct alone should lead to obstruction. The influence of the last-mentioned anatomic arrangement of the ducts, as well as the elevation of serum lipase activity which follows obstruction, are well illustrated in the following case:

REPORT OF CASES

CASE 1.—A married woman, aged forty-eight years, registered at the Mayo Clinic, Sept. 9, 1933, complaining of abdominal pain, jaundice, and loss of weight. In February, 1932, she had had diffuse, cramping pain in the abdomen that had lasted a few hours; this had been followed by a slight chill, the development of jaundice, and passage of clay-colored stools and dark urine. These symptoms had cleared within four days; two weeks later a similar episode had occurred. In October, 1932, four severe abdominal cramps, each lasting a half day, had occurred. These had been followed by severe chills, fever, and jaundice. The jaundice had fluctuated, but had remained until the end of December, 1932. During this time the stools had varied from black to clay color, and the patient had lost considerable weight and strength. Severe pruritus also had been noted.

The patient was emaciated and markedly jaundiced. The abdomen was moderately distended. Resistance to palpation in the right upper quadrant of the abdomen, and questionable ascites, were noted. The temperature was 103° F. and the pulse rate 120 beats per minute. The temperature fluctuated irregularly, varying from 99° to 103° F. Urinalysis repeatedly gave negative results, except for the presence of bile and occasional granular casts and pus cells. Serologic tests for syphilis gave negative results. The concentration of hemoglobin was 48 per cent (Dare); erythrocytes numbered 2,600,000, and leucocytes 17,800 per cubic millimeter of blood. Of the leucocytes 92.5 per cent were polymorphonuclears. The concentration of blood sugar was 83 mg. for each 100 c.c. of blood. Determinations of serum bilirubin and serum lipase may be noted in Fig. 1. The stools repeatedly gave negative results for bile, and intermittently gave positive results for blood. The test

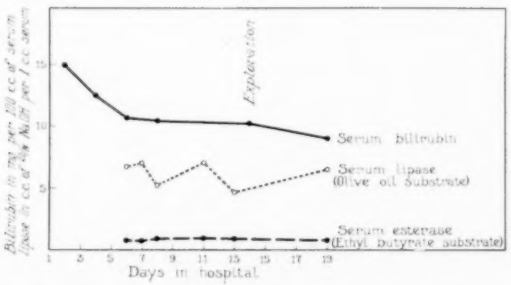

Fig. 1.—(Case 1.) The increased concentration of serum bilirubin and the serum lipase activity in obstruction of the pancreatic and common bile ducts caused by carcinoma of the ampulla of Vater. The esterase activity is not increased.

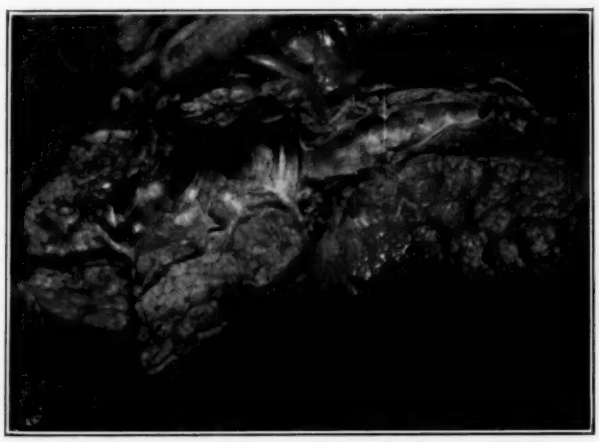

Fig. 2.—(Case 1.) Specimen obtained at necropsy; dilatation of the main and accessory pancreatic ducts secondary to obstruction by carcinoma of the ampulla of Vater may be noted.

for galactose tolerance was negative. The diagnosis was stone in the common bile duct, obstructive jaundice, and cholangeitis.

After the patient had been given solution of glucose and of calcium chloride intravenously, and a transfusion of blood, operation was performed September 22. Approximately 800 to 1,000 c.c. of turbid fluid escaped when the abdomen was opened. The liver contained multiple small carcinomatous masses. The gallbladder was distended, but its walls were normal in color and thickness. An indurated mass, approximately 5 cm. in diameter, at the head of the pancreas was found; this seemed to be carcinoma. The wound was closed after exploration. The postoperative course was not at all satisfactory, and the patient died on the sixth day after operation. The concentrations of serum bilirubin and serum lipase remained at a constant level following operation.

At necropsy the liver was somewhat enlarged, weighed 1,700 gm., and contained multiple carcinomatous masses. The gallbladder was dilated and contained approximately twenty stones; its walls appeared normal. The intrahepatic and extrahepatic bile ducts were markedly dilated; four small stones were found in the common bile duct, but were not large enough to obstruct the dilated duct. The pancreatic ducts were likewise dilated (Fig. 2), and the accessory duct ended in a blind pouch behind the common bile duct. The orifice of the ampulla was almost completely occluded by a polypoid mass measuring 1.5 by 1 by 1 cm. in diameter, which on microscopic examination proved to be a carcinoma. Metastasis had involved the regional lymph nodes. Microscopic examination of the pancreas revealed no evidence of infection.

Comment.—The obstruction and marked dilatation of the pancreatic duct (Fig. 2) was possible only because the accessory duct ended in a blind pouch and did not open into the duodenum. In dogs, complete obstruction is followed by prompt initial rise and subsequent disappearance of lipase from the serum. A secondary rise may occur. Since complete obstruction is not associated experimentally with prolonged elevation of the lipase values, the incompleteness of the obstruction probably accounts for the continued secretion and absorption of the pancreatic juice, and for the duration of the increased values in the blood stream.

Although little evidence of cholangeitis was found at necropsy, cholangeitis, as well as stones in the common bile duct, may possibly have played some part in causing the symptoms. Although clinically the picture simulated closely that of stone in the common bile duct, obstructive jaundice, and cholangeitis, the widespread metastasis that arose from a small carcinoma of the ampulla of Vater is indisputable evidence that the carcinoma had been present much longer than the last episode of jaundice, which had persisted for three months.

In 45 per cent of the cases of pancreatitis, in which the condition had been found at operation or at necropsy, the lipase values were higher than 2 c.c. of sodium hydroxide (per 1 c.c. of serum). In no instance was chronic disease associated with serum lipase values higher than 2 c.c. of serum. In only one instance did acute disease of the pancreas fail to produce activity higher than 2 c.c. In this case the disease was of a fulminating type, with almost complete destruction of the organ. Destruction of the finer blood and lymph vessels may have prevented absorption, and may explain the values obtained in this case. The elevation of the lipase values in association with degrees of pancreatitis not quite great enough to be more than suspected clinically, as in Case 2, is a more important observation.

Case 2.—A married woman, aged thirty-five years, registered at the clinic Nov. 9, 1933, complaining of attacks of abdominal pain and jaundice. In the last six years she had had recurrent attacks of colicky pain in the epigastrium, that had extended to the back, had lasted two or three days, and had occurred three or four times yearly. Between attacks there had been intolerance to cabbage, beans, and greasy food, with considerable gaseous dyspepsia and nausea. In the last two months the attacks had been more frequent and severe. October 24, a sudden, severe epigastric pain had occurred, which had extended to the right shoulder, had recurred for hours, had required morphine for relief, and had been associated with soreness in the right upper quadrant of the abdomen. October 28, jaundice had appeared, the stools had become dark and the urine light, and generalized pruritus had developed. Occasional, colicky pains had recurred, and the jaundice had fluctuated in degree.

At examination the jaundice was marked. Tenderness deep in the right upper quadrant could be elicited by pressure. A mass near the median line, thought to be the distended gallbladder, could be felt. Urinalysis repeatedly gave negative results, except for bile and granular casts. Examination of the blood and serologic tests for syphilis gave negative results. November 12, another colic occurred, followed by elevation of temperature to 101° F. and increase in the concentration of bilirubin from 10.2 to 16.2 mg. per 100 c.c. of serum. The lipase value was 10.2 c.c. (Fig. 3). The diagnosis was chronic, subacute cholecystitis with stones, stone in the common bile duct, obstructive jaundice, and pancreatitis.

Exploration, carried out two days later, revealed that the gallbladder was subacutely inflamed; its walls were thickened and edematous. Three stones, varying in size from 2.5 cm. to 3 cm., were removed from it. The head of the pancreas was enlarged and brawny, with diffuse inflammation of marked subacute pancreatitis. The common bile duct was opened and examined but no stones were found; in its walls was the same degree of inflammation as in the gallbladder. Whitish, puslike material was present in the bile. The scoop was passed into the duodenum easily. A T-tube was placed in the common bile duct. The liver had changed to almost greenish brown, and its lobules stood out as punctate spots.

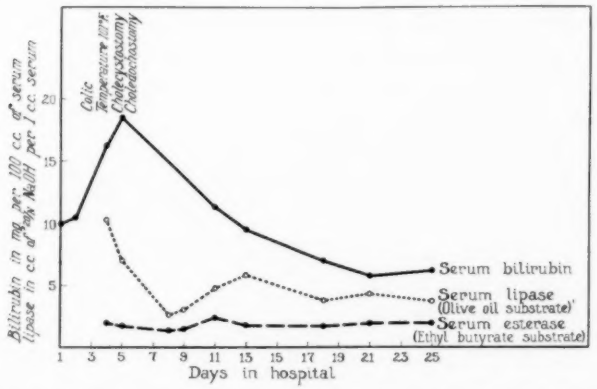

Fig. 3.—(Case 2.) The increased concentration of bilirubin and the serum lipase activity in cholangeitis, hepatitis, and pancreatitis. The serum lipase activity at its maximal during an attack of pain, chill, and fever, declined in a manner parallel to the concentration of serum bilirubin with the subsidence of the acuteness of the process. The esterase activity is not increased.

The postoperative course was satisfactory, with the exception of an elevation of temperature on the twelfth and thirteenth days.

Comment.—The rise in the lipase values following the attack of obstruction of the common bile duct and pancreatitis, as well as the fall in lipase values postoperatively (Fig. 3), well illustrate the influence of an acute process and its subsequent resolution on the serum lipase activity. The clinical symptoms did not point to pancreatitis. The preoperative diagnosis of pancreatitis depended entirely on the high activity of lipase in the serum. It is possible that the greatest sphere of usefulness of this lipase test of pancreatic function will be found in such cases, and in those in which mild attacks of epigastric distress occur without definite clinical, roentgenologic, or other laboratory evidence of cholecystic or pancreatic disease.

In neither of these two cases in which disturbance of the enzymatic function took place was there alteration in the islands of Langerhans sufficient to produce glycosuria or hyperglycemia. In only two cases of pancreatitis in the

series did such glycosuria appear. The lipase activity of the serum promises to be a more sensitive index of pancreatic dysfunction than glycosuria or hyperglycemia.

SUMMARY AND CONCLUSIONS

An enzyme capable of splitting simple esters (esterase) is normally present in the blood serum, but its activity bears no consistent relationship to any disease so far observed by us. An enzyme capable of splitting olive oil (lipase) usually is present in the serum of man. In 80 per cent of the cases the lipase activity in terms of twentieth normal sodium hydroxide is less than 2 c.c. (per 1 c.c. of serum). Although a greater number of patients without disease must be examined before a final statement can be made regarding the range of normal value for lipase activity, it appears that the upper limit is in the region of 2 c.c. (per 1 c.c. of serum). The activity of serum lipase was higher than 2 c.c. of twentieth normal sodium hydroxide (per 1 c.c. of serum) in 45 per cent of the cases of pancreatitis, and in 33 per cent of the cases of carcinoma of the head of the pancreas and of the ampulla of Vater. Such values were due to acute or subacute pancreatitis rather than to chronic pancreatitis, or to obstruction to the outflow of the pancreatic juice, due to carcinoma of the head of the pancreas. Lipase activity higher than 2 c.c. (per 1 c.c. of serum) was also encountered in eleven (6 per cent of the 162 patients) who had duodenitis, inoperable carcinoma of the stomach, carcinoma of the gallbladder, toxic hepatitis secondary to lobar pneumonia, or stone in the common bile duct with cholangeitis or peritonitis. It has been pointed out, however, that high values may not primarily be related to such diseases, but to associated pancreatic disease. Although further experience with increased serum lipase activity as a test of disturbed function of the pancreas is needed to establish the specificity of its relationship to that organ, the test appears at present to be good evidence of disturbed pancreatic function, especially when associated with the signs and symptoms of cholecystitis, choledocholithiasis, cholangeitis, and pancreatitis.

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THE PROGNOSIS OF CORONARY THROMBOSIS BASED ON THE NONPROTEIN NITROGEN IN THE BLOOD*

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NELLIS FOSTER[1] reported eight cases of heart disease in which the nonprotein nitrogen was elevated. Four cases of this group came to autopsy, and none showed evidence of nephritis. He suggested that an increase in the nonprotein nitrogen may be a resultant of purely circulatory disturbances. The cases reported by Foster and their respective filtrate nitrogen values are tabulated below.

ANATOMIC DIAGNOSIS	NONPROTEIN NITROGEN
(1) Cardiac, valvular	40 mg.
(2) Cardiac, mitral stenosis	59 mg.
(3) Cardiac, aortic insufficiency	61 mg.
(4) Cardiac, aortic insufficiency	47 mg.
(5) Cardiac, mitral stenosis (cerebral emb.)	53 mg.
(6) Cardiac, mitral and tricuspid	73 mg. and 85 mg.
(7) Cardiac, mitral and tricuspid	90 mg.
(8) Cardiac, aortic	43 mg.

He opined, "if these results can be confirmed by other students of these diseases the fact involved is important as indicating the part played by the circulation in a picture already recognized as composite."

In 1933 the author[2] reported sixteen cases of coronary thrombosis in which particular attention was paid to the nonprotein nitrogen. Ten of these reported cases showed a filtrate nitrogen of 40 mg. per 100 c.c. of blood or more. None of these cases showed evidence of a marked nephritis. It was also shown that even a marked drop in blood pressure and a diminution in urinary output were not sufficient in themselves to account for the nonprotein nitrogen rise in coronary thrombosis. During the past three years, the author has been able to collect thirty-one cases of heart disease due to coronary thrombosis in which particular attention has been paid to the nonprotein nitrogen value as regards its relationship to this interesting and more recently diagnosed heart disease.

In Table I, the author attempts to show in cross sectional study the relationship of nonprotein nitrogen, available kidney function test or urinalysis, and the time in which the coronary occlusion may have occurred. A cursory examination of this table will show that, whereas, in some cases the "pthalein" excretion and the nonprotein nitrogen value are in harmony, in other cases there are no such relationships. Still more important is the fact that the kidney shows ability to concentrate well in some cases in which the "pthalein" excretion is diminished. Sufficient cases are included in Table II to indicate that a drop

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Received for publication, May 11, 1934.

TABLE I*

NAME	AGE	DATES OF ATTACK OR ATTACKS	NONPROTEIN NITROGEN (MG. PER 100 C.C.)	KIDNEY FUNCTION TESTS	URINE
J.S.	59	9/28/33	40—10/ 2/33 31—10/ 5/33 38—10/10/33 27—10/13/33	†PSP—65% 10/4/33	10/2/33 Sp. G. 1020. Neg. alb. Occ. granular cast. 10/10/33 Sp. G. 1030. Occ. R.B.C.
M.B.	43	Minor anginal attacks for 2 yr. Typical attack 10/16/33	42—10/18/33 23—10/24/33 40—11/ 9/33 34— 1/13/34 34— 1/26/34 35— 2/ 2/34	PSP—45% 11/21/33	Urines neg. on many occasions. Sp. G. 1030 on 11/19/33. Tr. alb.
E.C.M.	52	Minor anginal attacks since Dec., 1930	44—12/ 3/31 50— 3/12/32 48— 3/14/32 50— 3/21/32 47— 6/ 7/32	PSP—52% 12/7/31 PSP—65% 3/19/32 PSP—30% 6/16/32 3/22/32 Sp. G. 1023 to 1027	10/7/31—Sp. G. 1022. Tr. alb.; occ. granular and hyaline cast. 3/13/32—Sp. G. 1023. Tr. alb.
R.S.	67	Minor anginal attacks for past 4 to 5 yr. Severe attack 10/17/33	36—10/20/33 41—10/23/33 38—10/26/33 32—10/31/33	PSP—40% 10/21/33	10/17/33 negative. 10/31/33 negative. 11/11/33—Sp. G. 1020; tr. alb., occ. R.B.C. Rare hyaline cast.
G.C.	47	Severe attack Jan., 1932, and May 5, 1933	150— 9/12/33	PSP—80% 2/22/30	9/12/33—Sp. G. 1020; tr. alb.
L.P.	42	Severe typical attack first and third weeks July, 1933	40— 7/26/33 30— 8/12/33 31— 8/19/33	PSP—50% 8/12/33	7/26/33—Sp. G. 1028; 3 R.B.C./HPF. Occ. epithelial and gran. cast.
A.M.	59	First attack June, 1930. Second attack Aug., 1933	33— 2/18/34 43— 2/20/34 37— 2/23/34 34— 2/27/34 40— 3/ 2/34 50— 3/ 6/34 52— 3/13/34	None	3/14/34—Sp. G. 1017. Two-plus alb. Neg. microscopic.
F.C.	30	First attack May, 1930; several minor attacks Aug. to Oct., 1930	33— 7/17/31 34—12/23/31 33—12/28/31	None	7/17/31 negative 10/25/31 negative 12/24/31 negative 5/ 8/32 negative
N.D.	51	Attack 5/22/33	23— 5/29/33 20— 6/ 5/33 19— 6/16/33	PSP—42% 6/22/33	Negative on several occasions
W.G.	49	Typical attacks 6/14/33 and 6/16/33	21— 6/17/33 32— 6/19/33 35— 6/22/33 30— 6/26/33 33— 6/29/33	PSP—33% 6/19/33 PSP—59% 6/29/33	6/18/33—Sp. G. 1020; Neg. except for few hyal. and gran. casts.
J.M.D.	65	Attacks August, 1932, and August, 1933	37— 8/22/33 35— 8/26/33 34— 8/29/33	None	8/25/33—Sp. G. 1023; 40 to 50 R.B.C./HPF.

*This table includes all cases that were not reported in entirety or at all in the author's preliminary report.

†This test in this paper denotes the two-hour intravenous method.

TABLE I—CONT'D

NAME	AGE	DATES OF ATTACK OR ATTACKS	NONPROTEIN NITROGEN (MG. PER 100 C.C.)	KIDNEY FUNCTION TESTS	URINE
M.M.M.	65	Minor anginal attacks for three years; severe attack 1/23/32	24— 1/25/32	None	1/25/32—Sp. G. 1025; V. Ft. Tr. albumin
M.M.	51	Severe typical attack 2/6/32; minor anginal attacks since	43— 2/15/32 39— 3/23/32	PSP—60% 2/15/32; Mosenthol 2/21/32 Sp. G. 1010 to 1025	2/14/32—Sp. G. 1012 negative
W.T.C.	71	Typical attack 4/21/33	44— 4/11/33 67— 4/22/33 34— 4/24/33 36— 4/28/33 28— 5/ 2/33 28— 5/12/33 24— 5/23/33	PSP—35% 4/26/33 PSP—70% 5/5/33	Frequent urinalyses negative
C.B.	58	Typical attack 1/14/33	39— 1/16/33 34— 1/23/33 35— 1/25/33 32— 1/27/33 200— 1/30/33	PSP—65% 1/23/33	1/2/33—Negative except for one-plus albumin

TABLE II

CASE	BLOOD PRESSURE (DATES)		NONPROTEIN NITROGEN	INTAKE	OUTPUT	
J.M.D.	8/20/33	140/100		700 c.c.	40 c.c.	(Aur. fibrillation)
	8/21/33	120/ 80		Not recorded		(Aur. fibrillation)
	8/22/33	70/ 60	37	1,280 c.c.	550 c.c.	(Aur. fibrillation)
	8/23/33	100/ 70		Not recorded		(Aur. fibrillation)
	8/24/33	100/ 80		1,640 c.c.	1,200 c.c.	Regular rhythm
	8/26/33	100/ 60	35	1,360 c.c.	750 c.c.	Regular rhythm
	8/28/33	Not obtained		1,240 c.c.	500 c.c.	Regular rhythm
	8/29/33	Not obtained		Incontinent		
	8/30/33	Not obtained		Incontinent		
W.G.	6/16/33	100/?		Suppression for 24 hr.		(Aur. fibrillation)
	6/17/33	94/ 80	21	1,460 c.c.	1,200 c.c.	(Aur. fibrillation)
	6/18/33	100/ 80		2,080 c.c.	800 c.c.	(Aur. fibrillation)
	6/19/33	100/ 76	32	2,830 c.c.	500 c.c.	(Aur. fibrillation)
	6/20/33	80/ 60		1,440 c.c.	1,000 c.c.	(Aur. fibrillation)
	6/21/33	94/ 70		2,400 c.c.	980 c.c.	(Aur. fibrillation)
	6/22/33	94/ 64	35	1,400 c.c.	800 c.c.	(Aur. fibrillation)
	6/23/33	100/ 60		2,440 c.c.	1,100 c.c.	(Aur. fibrillation)
	6/25/33	100/ 70	30 (6/26)	1,640 c.c.	850 c.c.	(Aur. fibrillation)
	6/28/33	102/ 60				
	6/29/33		33			
C.B.	1/14/33 (9:00 A.M.)	140/ 90				
	1/14/33 (7:20 P.M.)	110/ 80				
	1/15/33	100/ 80		2,490 c.c.	500 c.c.	
	1/16/33	88/ 65	39	2,950 c.c.	400 c.c.	(Aur. fibrillation)
	1/19/33	85/ 60		1,100 c.c.	650 c.c.	Normal rhythm
	1/20/33	75/ 55	35	2,300 c.c.	1,100 c.c.	(Quinidine)
	1/21/33	85/ 60		1,830 c.c.	880 c.c.	
	1/23/33	70/ 45	34	2,150 c.c.	600 c.c.	

This table indicates that a drop in blood pressure and a diminution in urinary output may not influence the filtrate nitrogen in acute coronary occlusion. Adson and Brown[6] have found that a drop in blood pressure in essential hypertension may not influence the vital function of the kidneys.

in blood pressure and a diminution in urinary output may not increase the nonprotein nitrogen in coronary thrombosis. In the remaining three tables (Tables III, IV, and V), complete follow-up studies on the thirty-one cases are given. (The author wishes to apologize for repetition from his preliminary report, but this was necessary in order to give a more complete and vivid picture of the value of repeated nonprotein nitrogen studies in this disease.)

A careful review of the latter three tables reveals that a rise in the nonprotein nitrogen value is very common in acute coronary occlusion, that an elevated value in the filtrate nitrogen (particularly if it remains elevated in serial studies) offers a very, very poor prognosis, and that a normal nonprotein nitrogen or one that recedes from a higher to a normal value is of better prognosis. Continued studies may not show such optimistic conclusions as to the prognostic value of repeated nonprotein nitrogen studies in this heart disease, but this second paper should offer a stimulus for further study in this field.

Electrocardiography.—Forty-one electrocardiographic tracings were made on twenty-eight cases of the total thirty-one. The following abnormalities were noted: (A) slurring or notching of the QRS complex in twenty-one tracings;

TABLE III

TABULAR STUDY OF CASES SHOWING A NONPROTEIN NITROGEN BELOW 40 MG. PER 100 C.C. BLOOD

CASE	NONPROTEIN NITROGEN (MG. PER 100 C.C.)	OUTCOME	DATE (LAST CHECK-UP)
A.G.	30— 8/17/31	Alive	Dec. 26, 1933
M.Mc.	33— 9/10/31	Alive	Dec. 20, 1933
A.P.	34—12/10/31	Alive	Dec. 20, 1933
F.C.	33— 7/17/31 34—12/23/31 33—12/28/31	Alive	Dec., 1933
N.D.	23— 5/29/33 20— 6/ 5/33 19— 6/16/33	Alive	Dec. 20, 1933
W.G.	21— 6/17/33 32— 6/19/33 35— 6/22/33 30— 6/26/33 33— 6/29/33	Alive	Nov., 1933
M.M.M.	24— 1/25/32	Died	July, 1932 (lived for 6 mo after occlusion)
S.L.	32— 1/26/31	Died	1/27/31
J.M.D.	37— 8/22/33 35— 8/26/33 34— 8/29/33	Died	Aug. 30, 1933
G.H.	37— 4/17/31	Died	April 25, 1931
C.B.	34— 1/ 8/34 31— 1/13/34 30— 1/16/34 38— 1/19/34 33— 1/26/34 30— 1/30/34 33— 2/ 2/34 33— 2/ 6/34	Alive	Feb. 12, 1934

TABLE IV

TABULAR STUDY OF CASES SHOWING AN ELEVATED NONPROTEIN NITROGEN

CASE	NONPROTEIN NITROGEN (MG. PER 100 C.C.)	OUTCOME	DATE
J.B.	48— 3/28/32	Died	May, 1932
S.A.	46— 1/ 2/31	Died	Feb. 6, 1931
C.P.	71— 8/ 3/31 95— 8/ 7/31 133— 8/10/31 185— 8/11/31 215— 8/14/31	Died	Aug. 14, 1931
J.T.	42—11/24/31	Died	Soon after leaving hospital, spring, 1931
B.W.	41— 3/ 6/32 40— 3/ 9/32 142— 3/28/32	Died	4/1/32
A.M.B.	60— 4/30/32 47— 5/ 3/32 40— 5/10/32 54— 6/10/32	Died	July, 1932
L.M.	40— 8/ 9/32	Died	Aug. 14, 1932
C.B.	39— 1/16/33 35— 1/20/33 34— 1/23/33 35— 1/25/33 32— 1/27/33 200— 1/30/33	Died	Jan. 30, 1933
E.C.M.	44—12/ 3/31 50— 3/12/32 48— 3/14/32 50— 3/21/32 47— 6/ 7/32	Died	June 22, 1932
G.C.	150— 9/12/33	Died	Sept. 14, 1933
M.M.	43— 2/15/32 39— 3/23/32	Died	Spring, 1932
A.M.	33— 2/18/34 43— 2/20/34 37— 2/23/34 34— 2/27/34 40— 3/ 2/34 50— 3/ 6/34 52— 3/13/34	Condition serious date of writing	

(B) ventricular premature contractions in six tracings; (C) auricular premature contractions in one case; (D) auricular fibrillation in seven tracings; (E) high take-off of the T-wave in three tracings; (F) an inverted T-wave in Lead I or II (or both) in thirteen tracings; (G) an upper convexity of the ST-interval in six tracings; (H) a deep Q_3 in five tracings; (I) left ventricular preponderance in eighteen tracings; (J) right ventricular preponderance in one case; (K) a delayed auricular-ventricular conduction time in one case; (L) and nodal rhythm in one case. In other words, there was evidence of coronary disease in twelve of the forty-one tracings. The history of viselike or crushing pain in the region of the precordium radiating down the left arm or up the left side of the neck plus a drop in blood pressure seems to be most common diagnostic criterion of coronary thrombosis. Only occasionally is the

TABLE V

TABULAR STUDY OF CASES SHOWING A REGRESSION OF NONPROTEIN NITROGEN

CASE	NONPROTEIN NITROGEN (MG. PER 100 C.C.)	OUTCOME	DATE (LAST CHECK-UP)
P.D.	75— 2/28/31 30— 3/16/31	Alive	Dec. 20, 1933
H.DeW.	110—11/27/31 42—11/30/31 38—12/ 4/31	Alive	Oct., 1933
S.S.	29—10/18/32 45—11/ 5/32 27—11/14/32	Alive	Dec., 1933
J.S.	40—10/ 2/33 31—10/ 5/33 38—10/10/33 27—10/13/33	Alive	Jan. 1, 1934
R.S.	36—10/20/33 41—10/23/33 38—10/26/33 32—10/31/33 35—11/ 3/33 32—11/ 8/33	Alive	Jan. 21, 1934
L.P	40— 7/26/33 30— 8/12/33 31— 8/19/33	Alive	Sept. 5, 1933
W.T.C.	44— 4/11/33 67— 4/22/33 34— 4/24/33 34— 4/26/33 36— 4/28/33 28— 5/ 2/33 27— 5/ 5/33 28— 5/12/33 24— 5/16/33 24— 5/23/33	Alive	Dec., 1933
M.B.	42—10/18/33 23—10/24/33 40—11/ 9/33 39—11/15/33 39— 1/ 8/34 34— 1/13/34 34— 1/16/34 34— 1/19/34 34— 1/26/34 35— 2/ 2/34	Alive	Feb. 12, 1934

electrocardiogram of value in the definite diagnosis of coronary thrombosis. At no time is it an emergency diagnostic procedure.

Serology.—The complement fixation test was negative in twenty-eight cases. The Kahn precipitation test was negative in the same twenty-eight patients. The serology was positive in the remaining three cases. The total percentage of syphilitic cases in this group was, therefore, 9.6 per cent. Syphilis, meaning positive Wassermann reactions, was noted in 4.5 per cent of Levine and Brown's cases,[3] and in 14 per cent of Conner and Holt's cases.[4] Warthin[5] believed that syphilis was a predisposing factor to coronary disease.

Comment.—Nellis Foster was probably the first observer to note that uremia might be associated with circulatory disturbances, and that the uremia may occur in such cases without nephritis. In his cases, he did not include coronary

occlusion. The author probably was the first observer to note that a high nonprotein nitrogen in the blood is very often associated with coronary thrombosis. In fact, a filtrate nitrogen value of 40 mg. or above was noted in twenty of the thirty-one cases reported in this paper.

SUMMARY

Thirty-one cases of heart disease due to coronary thrombosis are presented. The clinical history in all these cases was typical. No atypical cases are included in this study. (1) Uremia seems to be a common finding in coronary thrombosis. (2) Serial studies of the nonprotein nitrogen in the blood may offer an aid in prognosis. (3) A filtrate nitrogen that remains high or continues to rise is of ill omen, whereas, a lower nonprotein nitrogen or one that recedes is of much better prognosis. (4) Further studies in this interesting field are indicated.

The author wishes to express his gratitude to Dr. David A. Haller, Chief of the Medical Service, who has made this study possible. He thanks Dr. M. E. Missal for the complete record of case G. C.

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LABORATORY METHODS

A MODIFIED TECHNIC FOR STERNAL PUNCTURE AND ITS VALUE IN HEMATOLOGIC DIAGNOSIS*

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THE bone marrow is the largest organ in the body and comprises 4.6 per cent of the body weight.[1] It is the site of production of the erythrocytes, leucocytes, and platelets, and the cellular content of the peripheral blood reflects the changes going on in the parent tissue. In the past the study of blood dyscrasias was aided by sternal biopsy with the trephine. This procedure has to be done in the operating room, however, and is sometimes quite an ordeal for a sick patient. In addition, it cannot be repeated at frequent intervals, as is the case with sternal puncture.

Arinkin[2] was the first to use sternal puncture to determine the cellular composition of the marrow. Shortly after, Arjeff[3] devised a special needle to facilitate this technic. A detailed description of our method follows:

The site selected for puncture is in the middle of the sternum just below the juncture of the body with the second rib. A small spot 1 cm. in diameter is painted with iodine. The skin over this point is infiltrated with 1 per cent

Fig. 1.

novocaine, and the novocaine is then injected straight down to the sternum and the periosteum is infiltrated. This step is important to make the puncture painless. The marrow needle is similar in construction to that of Arjeff, except that its bore is 10 gauge and the guard can be lowered to within 0.2 cm. of the tip to make it available for children also. For adults the guard is set at 1 cm. from the point of the needle. To make the puncture, the sterile needle is pushed vertically through the spot prepared as above until it reaches the periosteum. If the outer plate of the sternum is not too thick, the needle can be pushed through by hand. If the resistance is too great a few gentle taps with a small mallet will suffice to drive the needle into the spongy bone. The stylet is removed and a sterile 20 c.c. record transfusion syringe is attached and suction applied. If the needle is deep enough bloody fluid will appear in the syringe. If not, the stylet is replaced, the guard raised a few turns, and the needle is driven in until blood can be sucked out. About 10 c.c. of the bloody fluid is

*From the Lenox Hill Hospital, and City Hospital.
Received for publication, February 24, 1934.

aspirated and mixed in a 15 c.c. centrifuge tube with 2 c.c. of a 1.4 per cent sodium oxalate solution. This has been shown to be an isotonic anticoagulant which does not change the volume of the cells.[4] The mixture is now centrifuged about five minutes at moderate speed. The supernatant plasma is removed, using a capillary pipette with a rubber nipple attached. By carefully manipulating the same pipette, the buffy coat is removed and transferred to a tube made by sealing off one end of a short piece of glass tubing of slightly larger diameter than the pipette. This is again centrifuged for five minutes and after the plasma is removed the buffy coat is pipetted off and smears are made on slides and stained as follows: Jenner stain, three minutes; distilled water, three minutes; decant; flood with dilute Giemsa, 1 drop to 1 c.c., thirty minutes; wash and dry. A differential count of 1,000 cells is then made.

The aforementioned authors[2, 3] made their smears directly from the aspirated sternal contents and a count of 1,000 cells was extremely tedious. The technic of concentrating the cells, as described here, has already been found of value in studying the peripheral blood.[5] Without distorting the cells, or interfering with their staining properties, this method permits a rapid survey of the sternal contents with an easy count of 1,000 cells. The normal differential count is approximately as follows:

Polymorphonuclear neutrophiles (mature)	25%
Polymorphonuclear neutrophiles (young forms)	5%
Polymorphonuclear neutrophiles (band forms)	10%
Lymphocytes	10%
Monocytes	1%
Eosinophiles	1%
Myeloblasts	2%
Premyelocytes and myelocytes	20%
Erythroblasts	23%
Proerythroblasts	2%
Plasma cells	1%

A few actual cases may be of value in illustrating the practical importance of this procedure:

CASE 1.—The patient was a white female, fifty-five years old. She was well up to three months before admission to the hospital, when she began to lose strength and complained of shortness of breath. On admission to the hospital, she looked acutely ill and had a temperature of 101°. She was very pale, the spleen was moderately enlarged and petechial hemorrhages were present in the mouth and over the skin. Her blood count was hemoglobin 40 per cent, R.B.C. 2.5 mil., W.B.C. 128,000. Differential count, neutrophiles 2 per cent, lymphocytes 8 per cent, myelocytes 1 per cent, myeloblasts ? 89 per cent. There was some doubt if these immature cells were myeloblasts. Marrow puncture showed erythroblasts 5 per cent, myeloblasts 95 per cent. The diagnosis was therefore definitely established as myeloblastic leucemia.

CASE 2.—The patient was a white female, seventy years old. For the past six months she had been losing strength and had become markedly anemic. She had also lost her appetite and had no desire for food. The tongue was somewhat atrophic and the skin lemon yellow in color. Gastric analysis revealed no free HCl. The blood count was hemoglobin 20 per cent, R.B.C. 1.0 mil., W.B.C. 9,800. Differential count, neutrophiles 63 per cent, lymphocytes 20 per cent, monocytes 15 per cent, eosinophiles 1 per cent, basophiles 1 per cent. The differential diagnosis was between pernicious anemia and gastric malignancy. Owing

to her weakened condition G.I. series was deferred. Bone marrow revealed, neutrophile polys (mature) 36 per cent, neutrophile polys (young forms) 3 per cent, neutrophile polys (band forms) 13 per cent, eosinophiles 1 per cent, lymphocytes 22 per cent, myeloblasts 2 per cent, premyelocytes and myelocytes 20 per cent, erythroblasts 1 per cent, plasma cells 2 per cent. A bone marrow showing only 1 per cent erythroblasts could not be that of a pernicious anemia. This fact was completely substantiated when x-ray revealed a carcinoma of the stomach.

CASE 3.—The patient was a white female, forty years old. The history was of three weeks' duration and the patient complained of marked prostration and of a severe ulceration of the mouth. The temperature was 100.5°. The blood count was hemoglobin 80 per cent, R.B.C. 4.4 mil., W.B.C. 1,000. Differential count, neutrophiles 23 per cent, lymphocytes 73 per cent, monocytes 4 per cent. The diagnosis was agranulocytic angina with a possibility of a leucopenic lymphatic leucemia. Sternal puncture showed neutrophile polys (mature) 22 per cent, neutrophile polys (young) 6 per cent, neutrophile polys (band forms) 16 per cent, lymphocytes 10 per cent, myeloblasts 2 per cent, myelocytes and premyelocytes 21 per cent, erythroblasts 21 per cent, proerythroblasts 1 per cent, plasma cells 1 per cent. In this case a study of the marrow by sternal puncture not only definitely ruled out a lymphatic leucemia, but showed that this was a case of agranulocytosis with maturation arrest, rather than marrow aplasia.

It is therefore seen that the above described method of sternal puncture is simple, readily adaptable for routine hospital use, and is of real value in getting additional information in the study of the blood dyscrasias.

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COMMENTS AND PROCEDURE ON THICK BLOOD FILM TECHNIC*

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THE advent of the thick-film method for the examination of dried blood films that combined laking and staining in one step was a fine advance in laboratory diagnosis. Barber and Komp's[1, 2, 3] modifications to improve the quality of the preparations and simplify the mechanical handling of the slides for mass rural survey work was another distinct advance in technic. This is an excellent time-saving method for community work and can be applied equally well to surveys of domestic and wild animals if confined to mammalian life.[2, 3] The benefits derived from the relatively new method certainly invite a much wider use than at present prevails among field, clinical and research workers. This is particularly true for those who are located in tropical regions. It permits the use of four or five times as much blood as can be made into a satisfactory thin film and reduces the searching time from about thirty minutes to three or five minutes. A vast number of large parasites such as microfilaria, trypanosomes and even the large forms of malaria parasites are carried away by the instrument used in spreading a thin film. None of the blood taken by the thick-film method need be lost by the use of a spreading or stirring instrument. Cycles in the chronic stage of a disease can be more easily followed. It is even helpful in preliminary diagnostic work in revealing the presence of leucemia, leucopenia, leucocytosis, eosinophilia and polychromatophilia. The writer recently found a case of myelogenous leucemia during the course of a survey for malaria. This man had not been under treatment and was not aware that he had a condition that warranted attention.

An experienced technician can examine with a fair degree of accuracy 125 thick films in seven hours provided that the films are well prepared and do not exceed a half inch in diameter. The writer's experience covers twelve years in the tropics and four years of that time have been very largely spent in thick, blood film work. This personal experience with the method is not given with the intention of matching it with others who may already employ the method but rather to stimulate fellow technicians in its use and to mention the latitude the technic permits in its application.

Slides.—One should be sure that all slides fit the slide boxes. Frequently, they are too long or too short or even too thick. Very thin slides offer trouble with the use of the mechanical stage. We use green tinted slides rather than the colorless or clear slides. They are capable of longer use and stand cleaning better.

*From the Gorgas Memorial Laboratory.
Received for publication, February 20, 1934.

New slides should be cleaned and soaked in alcohol before use, and it is very necessary to have them perfectly dry and free of dust as well as fingerprint grease.

Used survey slides should have the cedar oil and stained blood film removed as far as possible by rubbing them with gauze moistened with xylol before they are immersed in a hot (not boiling) solution of a mild soap. The slides are then washed individually in running water and then immersed in 70 per cent alcohol overnight. They should be dried with a clean gauze cloth and placed in the standard cardboard shipping boxes lined with gauze. Dust from the cardboard will collect on them after long travel in boats, cars, or on mules unless this measure is taken.

Equipment.—The equipment required depends on whether it is intended for hospital or field work. None is needed for hospital work except that an extra slide should be taken on which to make the film. One large drop of

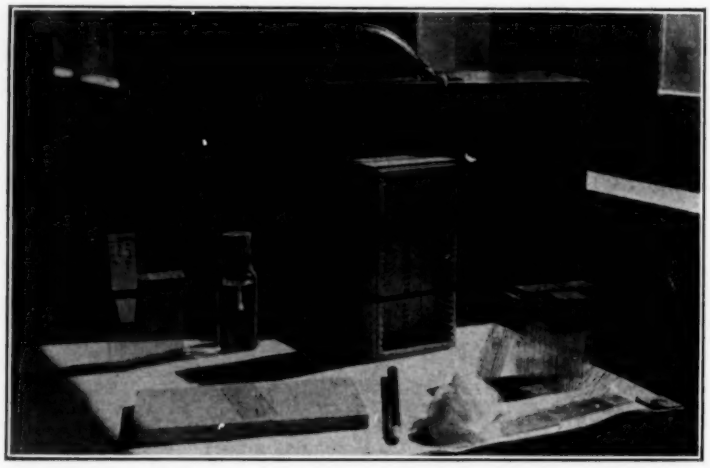

Fig. 1.—A view of the slide box and its stand, the field case, the needle bottle and a block of slides with the identification tag.

blood can be maneuvered into a circular spot about half an inch in diameter at one end of the slide. This must dry thoroughly without causing irregularity in the film margin else time is lost in examining the protrusions that form. The film must be at least a quarter of an inch away from the end of the slide to permit the mechanical stage to cover the entire film without interference from the objective. The only thing that fastens this film to the slide is thorough drying. The slide is stained standing on end to save staining solution and prevent the collection of débris on the film.

Field equipment must be assembled to meet the needs for the day. One must have some knowledge of how many people or animals are to be included in a survey in order to provide sufficient slides. In the case of people, squares of gauze and a bottle of 70 per cent alcohol are needed to clean the skin of the ear, finger, or toe from which blood is to be taken. A two-ounce bottle of alcohol with a No. 5 Hagedorn needle inserted in the cork makes a good blood-lancet. Each time the cork is replaced the needle is submerged in

alcohol thus preparing it for the next person. One should dip the blood-lancet needle up to the cork in paraffin to prevent rusting if it is not in daily use. A wax pencil is required to number the slide on the end not occupied by the film. A lead pencil is wanted to record these numbers in a field book with the name, age, etc., to follow.

Slide boxes with a capacity of 25 slides are used and on these boxes are entered the village, school or organization with the first and last numbered slides. These boxes should be held tightly closed by an elastic band and kept standing in a vertical manner until the blood films are quite dry. To do this, while a slide box is being filled, I use a right-angled stand made of blocks two inches thick. The upright block is the same height and width of a slide box. Another block 4 inches long and the same thickness and width is nailed to the bottom of the upright piece. This makes the base that rests on the table or stand. The opened slide box is then strapped to the upright by an elastic band. This keeps the box level so the films can dry without running. On removing the slide box from this stand it is placed in the same vertical position in the carrying case.

Field Case.—The use of leather cases has been abandoned, since the field cases are subjected to all manner of transportation and to heavy rains. This laboratory uses a metal case 8 by 9 by 16 inches with a leather handle similar to that of a suitcase. This case will hold all equipment needed for 400 people or animals. These cases are really carpenter's tool kits made by the Kennedy Manufacturing Co. at Van Wert, Ohio, and Chicago, Ill. They come with a tray but we discard it. These cases are very much less expensive than any made of other materials and they stand severe usage as well as being rain-proof.

Animal Surveys.—The only variation in field equipment for these surveys is the addition of a scissors to clip hair off the point of an ear, or to expose the tip of a tail in small animals. A scalpel is also added. This is drawn lightly across the point of the denuded ear and then by pressure drops of fairly clean blood can be removed by contacting the slide. Always wipe the scalpel with alcohol before use on the next animal and apply iodine to the ear wounds. In some animals like the sloth and bat, a toe nail or thumb nail must be cut off to secure blood.

Source of Blood for Film.—There is some divergence of opinion as to the most suitable place from which to draw the blood. The writer, for field work, prefers the lobe of the ear as being less sensitive, cleaner, out of sight of the patient and as having some degree of capillary stasis. Parasitized cells are believed to be more abundant in such a system of capillaries. When very young babies are to be included in a survey, it is better to use the ball of the great toe. Many use, as a routine measure, the skin near the base of a finger nail. The Daland's blood-lancet is a very dissembling little instrument that does not necessarily require sterilization. It can easily be covered up with the same gauze used for cleansing the area to be punctured and the puncture is made with a firm quick stab without the patient knowing what moment it is to occur. This is an important point in the management of

nervous people. I have used this lancet on over 6,000 patients without the occurrence of local infection. Care should always be taken to dry the skin and to clear the puncture of any alcohol since blood expressed through or over an alcohol wet surface will fix the cells, and they will not lake as thoroughly as they should. A dry skin allows discrete drops of blood to form at the site of the puncture, and these are sure to be cleaner than blood rubbed off the skin. The blood specimen is stirred or maneuvered into a circular spot about the size of a dime. It is then given its place in the slide box and dried thoroughly before the vertical position of the box is changed. The field notebook will receive the same number given the slide and identification data will be entered on the same line with the number. If care is taken to secure a large drop of blood, there will be no need to stir it into a spot the size of a dime, since it will spread to the proper size if given a level position. Some place the slides with the blood film upside down in the box and place the wax pencil number on the other side at the opposite end. I like the number and the film on the same side of the slide but, of course, at opposite ends. The blood film end of the slide should always occupy the same side of the slide box else it will be difficult to block the slides in 25 lots for the staining bath. Much time is saved and better results are obtained if sufficient blood has been spread in a uniform manner and in a discrete spot about half an inch in diameter. Search the entire film, do not depend on the *thickest focus in the film to always supply the information you seek.*

When a slide box is filled, it is covered and the lid bound on the box with a good rubber band. The necessary information is written on the side or bottom of the box with a lead pencil. For example: Santa Rosa School, slides 1 to 25 or Santa Rosa School, slides 26 to 50, etc. These boxes are kept in a vertical position in the field case until the close of the day or until the following day. It is always better to stain them within twenty-four hours, since they lake with increasing difficulty after that period of time. We have, of course, used such films even after a month or two of drying but they are not as satisfactory and the blood film is apt to crack in many lines, and segments of the film may even be lost. I have been able to use films that were six months old but they are not desirable films. Animal surveys are frequently done in barns and whether the floor is of dirt or boards it pays to wet down the floor to avoid dust settling in the blood films. It is even more necessary to avoid destruction of the films by flies, ants, etc. Flies can remove a large part of a film in a very short time. Care must be taken to guard against breakage or leakage of the alcohol and iodine bottles carried in the field case, since a whole day's collection may be ruined for the staining and examination. Stock bottles are protected by several vertical and transverse rubber bands which not only serve to keep the corks in a tight position but also protect against the jarring of the bottles against each other during transportation.

Thick blood films will dry in a half hour during the *dry season* but in the *rainy season* it is always better to dry them a half hour by placing the closed boxes in the incubator at 37° C. The lid of a box is now removed and pieces of cardboard one inch square by 1/16 of an inch thick are dropped in the spaces between the slides on the ends where the identification numbers have

been written. It is well to drop one between the box and the first slide as well as between the last slide and the box. These cardboard separators are to provide a staining space between the slides. The lid is now replaced and the box turned wrong side up on the table, the bottom of the box is raised slowly taking note that no slides are fast in their brackets. The slides are then brought compactly together by a thumb and finger and moved to the side of the lid where the separators are then pushed flush with the ends of the slides. This is best done by lightly holding the block of slides while a scalpel blade pushes all separators into good position. These separators add protection to the numbers unless too firm a grip is used while moving them into position. In such a case they smear the wax pencil numbers and make reading difficult. The block of 25 slides is then lifted up to the edge of the lid while a good elastic band is placed about the numbered ends of the slides. A second band should then be applied over the first to guard against the breaking of the one band and the unfortunate release of the block of slides. Some prefer a rubber web for this instead of pure rubber bands. Rubber bands 3½ inches long by ¼ inch wide are used in our equipment. They are long enough to allow two turns of the band and the firm pressure is desired. This makes a solid block that can be handled with ease and safety. Beneath the rubber bands, a small strip of white card is adjusted and on this card is transcribed the entry made on the slide box such as: Santa Rosa School, 1 to 25. Once the boxes have all been blocked in this manner the blocked slides are ready to be stained.

Staining Method.—Giemsa's stain is probably universally used. It is an expensive stain when purchased ready for use and after long periods of storage some of the bottles of stain deteriorate. Its preparation from the powder is so simple, the results obtained so satisfactory and its price so greatly reduced that I see no reason why the stain should not be prepared in any laboratory. We estimate it at 75 cents an ounce (stock stain) when made by us. This laboratory does not use Azur II as is the case with the old formula. I place 2.4 gm. of Azur II eosin (Grübler) in a clean Erlenmeyer flask and then add 200 c.c. of C. P. anhydrous glycerin. The flask is covered with a well-fitted cork and then placed in a water-bath at 60° C. for thirty minutes. The flask is shaken once or twice during this period. It is then removed from the bath and 200 c.c. of C. P. methylic alcohol are added and after mixing the contents the flask is again placed in the hot water-bath for another half hour, shaking it once or twice during this period. It is then removed and left in an incubator overnight at 37° C. Next day it is passed through filter paper and stored in 200 c.c. bottles that have been thoroughly cleansed, washed with methylic alcohol, and dried before use. These are well stoppered and kept in the dark. I use equal parts of glycerin and alcohol rather than the larger amount of alcohol advised by some. The liberal use of alcohol is undesirable since thorough laking of the cells is the main object in thick film work. Everything used in the preparation of a stock of Giemsa stain should be as clean as the stock bottles in which it is stored. I use a set of glassware exclusively for this work. Application of the stain requires a dilution of this Giemsa stock

in a proportion of 1 c.c. to one ounce of water. Pour this mixture back and forth about four times and then consider the solution ready for use. The blocked slides are placed in the staining dishes resting on the film ends of the slides with a quantity of stain just sufficient to cover the films. Our service uses the rectangular staining dish 34 by 59 by 82 mm. inside measurement and these hold 3½ ounces of the staining solution and will receive 50 slides. White enameled trays 1⅛ by 2⅞ by 7½ inches (inside bottom dimensions) will accommodate 150 slides and use 9 ounces of staining solution. The slides are left in the stain for one and one-half hours, and then very gently dipped in two changes of water like that used for making the staining solution. They are then dried by standing the blocks of slides on paper that absorb water rapidly such as paper towels. We usually leave them overnight but drying can be hastened if necessary. The films must be thoroughly dry before they are examined. The authors[1, 2, 3] of the modified thick-film method that this

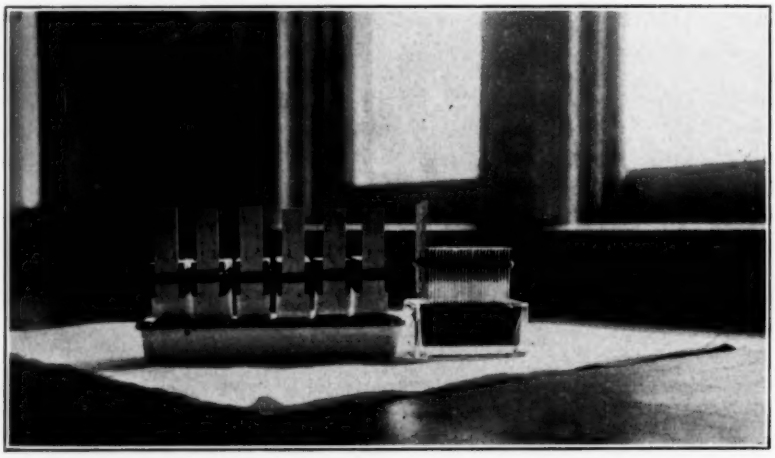

Fig. 2.—An instrument tray with 150 slides and the smaller staining dish with 50 slides in the aqueous solution of Giemsa's stain.

laboratory uses state clearly that the water used for diluting Giemsa stain should be neutral, or only slightly alkaline (pH 7.0 to pH 7.2), and must be nearly or quite free from salts. It has been our experience that clean, fresh rain water and our local city water supply all serve well without treatment for this purpose. Freshly distilled water needs to be corrected. There is always the risk that some accident or repair to the water supply system might cause the loss of a day's collection of slides but thus far it has not and I like our results as well as with water corrected to the proper values. There is considerable latitude in the character of the water to be used but one must stay within reasonable bounds. Much discussion has arisen from the formation of greenish yellow crystals that occasionally cover the film and make examination difficult. This accident will not happen if the staining dishes are kept thoroughly clean between their periods of use.

The complete examination by a trained technician of a well-stained thick film a half inch in diameter will require about three and one-fourth minutes.

There are three factors, at least, that must be taken into consideration in an efficient examination of a thick film, these are as follows: (1) one must be acquainted with the parasites for which he is searching, (2) the entire film should be searched, (3) a proper light and magnification must be employed and a sufficient amount of good immersion oil (cedar oil) to cover the search must be applied to the film.

One familar with thin films stained with an alcohol polychrome stain will find the thick film picture confusing for a time since all the red blood cells are gone. The field contains leucocytes, platelets, shadows of red cells, and parasites. It will not take much time for one who knows the parasites and is acquainted with thin film work to learn the thick film method, and for diagnostic work it is a most desirable method. There is more upon which to base an opinion than the sparse findings of a thin film. Both methods have their particular points of value but for mass survey work the thick-film method of today fills a most important need. More mixed infestations of malaria, scanty trypanosomal and spirochetal carriers are being found and in wild animal life it is uncovering many facts that are desired in the way of the incidence of filariasis,[4] trypanosomiasis,[5] spirochetosis,[6] piroplasmosis, anaplasmosis, etc. Naturally the different species of parasites vary a great deal in the number that may be found in a film. The monkey spirochete at the apex of an acute attack may show a countless number to a microscopic field while a trypanosome like *T. cruzi* may never reach a figure above 40 to a field and certain microfilaria may show but a few to the entire film. Scant infections are far more frequently found with this film technic. The writer and his fellow technicians have, during the past four years, examined from man and animals about 200,000 thick films. This diagnostic method is capable of just as wide use in veterinary medicine as it is in human medicine and affords an excellent yardstick to measure the results of sanitation and malaria control. The chief purpose of this thick film method is the diagnosis of parasites in mammalian blood. It is not intended for a study of the characteristics of these parasites or their relationship to the red blood cells.

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THE BIOLOGIC DIAGNOSIS OF TERATOMA TESTIS*

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IT HAS been shown by several workers, Zondek,[1] Heidrich and Fels,[2] Kantrowitz,[3] Lilienthal,[4] Paschkis,[5] Hady,[6] Bollag,[7] Weinstein and Schofield,[8] Johnson and Hall[9] and Ferguson[10] that the sex hormones prolan A and prolan B appear in the urine of men having teratoma testis. Of the above cited references all but Zondek and Ferguson are single case reports. Ferguson[10] classifies the malignant tumors of the testis that may cause the appearance in the urine of man of the prolan hormones as follows: (A) chorionepithelioma, (B) embryonal adenocarcinoma, (C) embryonal carcinoma with lymphoid stroma, (D) seminoma and (E) teratoma with adult features; the amount of hormone in these groups decreasing in the order given.

The work of Ferguson[10] indicates that a suitable test for the quantitative existence of these hormones in the urine is possible. At the time of his report we were employing rabbits in a similar test with somewhat similar results. The use of immature virgin female mice as employed by Ferguson simplifies the problem of handling, feeding and injecting animals. Employing urine from the same specimen and appropriately injecting a series of rabbits and a series of mice, we found from 22 to 25 mouse units as the equivalent to a minimal effective rabbit dose. Due to the advantages of using the smaller animal we now employ mice for the routine tests and reserve the rabbits for quick approximation when time is an important factor. In all cases the quantitative mouse test should be made.

PREPARATIONS AND TESTS

The diagnosis of pregnancy by means of the Aschheim-Zondek reaction is discussed in many textbooks and the methods of conducting the test are well known. The test as conducted for pregnancy, however, while essentially similar to that for teratoma, does not offer a quantitative value as to the amount of hormone or hormones in the urine. A quantitative test in teratoma is essential in order to obtain consecutive readings which will indicate a decrease or increase in the amount of hormone present when subsequent urine samples are tested.

Fresh morning urine and extracts of urine prepared after the methods of Zondek are employed. Injections are made intravenously in rabbits and intraperitoneally in mice. The extracts employed were made as follows: 50 c.c. of urine acidified to litmus by the use of acetic acid are placed in a precipitating jar and 250 c.c. of 95 per cent ethyl alcohol are added. The mixture is set in the ice box overnight and the resultant precipitate is obtained by centrifugation. The precipitate is then washed with about 50 c.c. of oxide-free ether.

*Published with the permission of the medical director of the Veterans Administration, who assumes no responsibility for the opinions expressed or the conclusions drawn by the writer.
Received for publication, March 2, 1934.

The precipitate is again obtained by centrifugation and decantation and is allowed to dry. The dry precipitate is then taken up in an aliquot part of water. In our work we commonly use 5 or 10 c.c. of distilled water; thus we have an extract that represents either a concentration of 10 or of 5. Not all of the precipitate is soluble in the distilled water, and it is customary to shake the watery suspension well and allow it to stand at least an hour but preferably overnight in the ice box, after which the residual precipitate may be removed from the watery solution by centrifugation. The supernatant fluid is then labelled and is ready for use.

Immature, virgin, female mice are employed as follows: six animals are selected and tagged or otherwise marked 1 to 6. Mouse number one receives 0.1 c.c. of fresh urine in each of 5 doses spaced over a period of forty-eight hours. Mouse 2 receives 0.2 c.c. of fresh urine in each of 5 doses which are similarly spaced. Mouse 3 receives 0.4 c.c. of fresh urine in each of 5 similarly spaced doses. Mouse 4 gets 5 doses of the 5x extract, 0.1 c.c. per dose. Mouse 5 receives five 0.2 c.c. doses of the 5x extract. Mouse 6 gets five 0.4 c.c. doses of the 5x extract. The 5 doses of extract are spaced over a forty-eight-hour period also. This gives a range in total dosage from ½ c.c. of fresh urine to 2 c.c. of the extract, and if the 5x extract is used, the highest dosage represents 10 c.c. of urine.

Readings are made on the ovaries about ninety hours from the first injection time. The ovaries are observed grossly and then removed for histologic section preparation. The sections provide us with a reference slide when subsequent tests are made on the same patient. The observations from the gross observations and from the sections are tabulated and translated into mouse units for purposes of record. The following description will explain how this is accomplished; the ovarian changes occurring in the test animals when injected with urine from teratoma patients are: first, a swelling and hyperemia of the graafian follicle accompanied by the formation of a distinct cumulus oophorus. Second, the formation of a massive hemorrhage within the ripened follicle, the so-called corpora hemorrhagica. Third, the formation of corpora lutea atretia. This latter reaction is quite easily distinguished under higher magnifications. Prolan A is said to be responsible for the first two reactions while prolan B is said to cause luteinization. Zondek[1] has shown that prolan A and prolan B exist in the urine of pregnant women in the ratio of 5 to 1. Ferguson[10] has reported that this ratio also holds for urine from males having teratoma. Our work also has shown that whenever Reaction 3 (corpus luteum) occurs, it is possible to get Reactions 1 and 2 with one-fifth to one-sixth as much urine.

The quantitative interpretation of results is as follows: positive cumulus oophorus formation with corpus hemorrhagica in Mice 1 to 6 indicate respectively 2,000, 1,000, 500, 400, 200, and 100 mouse units of hormone per liter of urine tested. It is not essential that corpus hemorrhagica be seen, as this reaction is not of lasting caliber. Reactions which represent cumulus oophorus formation, corpus hemorrhagica formation and corpus lutea atretia when occurring in Mice 1 to 6 indicate in respective order the following number of mouse units, 10,000, 5,000, 2,500, 2,000, 1,000, and 500.

The same reactions are observed in rabbits when 22 to 25 times as much urine or its extract equivalent is injected intravenously. Readings on the rabbit ovaries can be made twenty-four hours following the injection and but one single injection is employed in the rabbit test. The test is done in a manner similar to the Friedman modification of the Aschheim-Zondek test for pregnancy. If but one rabbit is employed for each patient, then the result will only indicate the presence or absence of from 22 to 25 mouse units of hormone in the dosage given, since the intravenous dosage would rarely be over 20 c.c. of urine. The test has its limitations even as an index as will be seen from Table I.

TABLE I

TEST RESULTS

RABBIT-MOUSE CORRELATION VALUES INDICATING LIMITATION OF RABBIT TEST AS TO DOSAGE EMPLOYED

CASE	RABBIT TEST	MOUSE TEST (MOUSE UNITS)
A	Negative	500
B	Negative	200
C	Questionable	2,000
D	Negative	1,000
E	Negative	2,000
F	Negative	500
G	Positive	2,500
H	Not run	1,000
I	Negative	100
J	Not run	1,500
K	Not run	2,000
L	Not run	2,000
M	Not run	200
N	Not run	Neg.
O	Positive	16,000

Rabbit test in this series all by single intravenous injections of 10 c.c. of fresh urine. Mouse units are calculated to liter of urine.

To compensate for the units of hormone per liter, the twenty-four-hour specimen volume is obtained and if any great variance in volume exists in subsequent tests on the same patient the hormone unitage total is adequately corrected. It is believed from the results that the amount of hormone remains fairly constant, tending to increase, of course, in untreated patients, regardless of the urine volume per day.

Occasionally patients with teratoma subjected to the test will show more than 10,000 mouse units per liter, viz., in Case 10. One single injection of 0.3 c.c. of urine produced all three reactions in a mouse. The interpretation here was at least 16,000 mouse units per liter of urine. A grave prognosis was given in this case. Before effective treatment could be employed or another test obtained, the patient had succumbed to the disease. This patient reported after considerable metastases had occurred and was critically ill at the time of admission. Autopsy confirmed the extensive metastatic involvement suggested by the test.

RESULTS OF THE TEST

A few case summaries are included to show the applications of the test. At present over 40 cases of teratoma are being followed by means of the test.

Since it has been shown by Zondek and by others that prolan A and prolan B do not occur in normal male urine, the observance of any of the three reactions mentioned are significant when obtained with male urine injections into either rabbits or mice.

Irradiation of the teratoma mass or of metastases from it reduces definitely the amounts of prolan A and prolan B in the urine, therefore the test has a use in diagnosis. Not only will the increases or decreases of hormone be evident in properly conducted tests but the effectiveness of treatment may be followed accurately. Postoperative tests will show if secondary areas are present. If such areas exist, then the test will indicate whether postoperative irradiation has effectively removed danger. From the cases listed and others, it is believed that if the test is positive preoperatively, rather strenuous irradiation should be applied before operative removal of such tumors. Most of the cases presented to us have had previous treatment, either surgery or irradiation but generally surgery. In these and in other cases the recognition of the presence of metastatic nodules, when such growths are not palpable, is extremely important.

FOLLOW-UP SYSTEM

A study of our records reveals the facts that a great many of our teratoma patients live great distances from the facility. It has been difficult to get such patients to report regularly for checking of physical condition and examination, and of course, under such circumstances it is difficult to control treatment. Our present system of follow-up on such patients now includes a mailing tube system for preserved urine specimens. We are attempting to obtain tests on all teratoma patients at least every two months. Patients at a distance from the hospital merely send by mail a sample of urine in a special case which is provided for the purpose. These preserved urine samples are then tested and the test results reviewed by members of the Tumor Clinic who recommend appropriate treatment or subsequent follow-up. Good cooperation on the part of the patients has been the result of this system. Considerable economy is also effected by employing the system, for often transportation expenses can be saved, and naturally hospitalization is not essential for patients with consistently negative results by the test. Since it is commonly accepted that the correct diagnosis of teratoma is made in less than 15 per cent of such cases presented for observation, it is felt that the test applied as a routine procedure in such suspected cases would result in improvement in the prognosis and number of cures by either irradiation or surgery or both.

Following irradiation or surgery, the test is often questionable until ten days or two weeks after the procedure; supposedly the hormones are not at once flushed from the system or the pituitary ceases the overproduction of them immediately. If the irradiation or other treatment is effective, however, the test when made two weeks after such treatment shows this by a reduced amount of hormone in the urine. Case 8 presents such a condition where the test was run too soon after the orchidectomy; a subsequent test in this case was recommended so as to check the result.

SUMMARY

1. Confirmation of Ferguson's results on the biologic testing for teratoma is offered.

2. A method for follow-up on such teratoma patients is presented.

3. Ten case summaries from a group of 40 teratoma cases are offered as typical examples of the test applications.

4. The importance of preoperative irradiation is stressed.

5. The necessity of consecutive checking, by the test, of such patients having teratoma is outlined.

CASE SUMMARIES

CASE 1.—White, aged thirty-seven. Injured left testicle in 1925 and again in 1929. Left orchidectomy in 1933 at another hospital. Histologic diagnosis: adenocarcinoma. No palpable glands nor abdominal masses on admission here. Deep x-ray to stump, abdomen, and mediastinum followed by a prolan test which showed 500 M.U. Repeat prolan test two months later gave 200 M.U.

CASE 2.—White, aged thirty-four. No history of injury. Gradual swelling of left testicle up to February, 1933, when a left orchidectomy was done at another hospital. Histologic diagnosis: cancer. Entered here March, 1933, with drainage from scar, inguinal glands palpable. Prolan test showed 1,000 M.U. Deep x-ray therapy to the abdomen and inguinal gland region. Subsequent prolan test was 500 M.U. Further treatment by deep x-ray to the mediastinum was given on suspicion. Supraclavicular area treatment refused.

CASE 3.—White, aged forty-four. No history of injury. About three years ago noted swelling of left testicle. In June, 1932, left orchidectomy at another hospital. Weight loss at the time was about 30 pounds. Transferred here for further treatment. Mass in upper left quadrant of abdomen, no other adenopathy. Chest was negative. Deep x-ray to the left scrotal stump and abdomen was given. Subsequent prolan test 3,000 M. U. Treatment by x-ray at a later date followed by prolan test of 2,000 M.U.

CASE 4.—White, aged forty-five. About ten years ago, had injury to right testicle; swelling resulted which subsided until a year ago when swelling of the right testicle again became apparent. Back pains, pulling sensations in right groin. Right orchidectomy at another hospital in February, 1933. No irradiation was given at the time. On admission here was given deep x-ray therapy to right scrotal stump and to abdomen. The prolan test following treatment was 2,000 M.U. on January, 1934.

CASE 5.—White, aged thirty-nine. History of testicular injury in June, 1933. In March, 1933, biopsy at another hospital showed suspected malignancy. Right orchidectomy was done at the same outside hospital. On admission here in April, 1933, he was given deep x-ray to the stump and abdomen and released. On readmission in August, 1933, no adenopathy was apparent. On another admission in October, 1933, the prolan test was negative. The follow-up specimen of January, 1933, shows 200 M.U. by prolan test.

CASE 6.—White, aged fifty-three. Injured left testicle in service; swelling followed by receding at that time. May, 1932, swelling of left testicle prompted hospitalization in August, 1932. In this outside hospital the scrotum was tapped and a left orchidectomy was done. Histologic diagnosis was carcinoma of testes. No palpable glands. Admitted here September, 1932. No palpable glands were noted. Irradiation to scrotal stump and abdomen. Follow-up specimen of January, 1934, shows 1,000 M.U.

CASE 7.—White, aged forty. Injury to right testicle on January, 1932, swelling continued. Admitted to another hospital where right orchidectomy was done November, 1932, with no other treatment. Admitted here January, 1933. Adenopathy negative, chest qua-

tionable. Irradiation deep x-ray to scrotal stump, abdomen, and mediastinum. Readmitted August, 1933. Irradiation to mediastinum over suspected chest nodes. Follow-up specimen of January, 1934, shows 1,500 M.U. by prolan test.

CASE 8.—White, aged thirty-four. No history of injury. December, 1932, gradual swelling of left testicle. December, 1933, left orchidectomy at another hospital with no other treatment. Admitted here December, 1933, where no adenopathy was found and irradiation was not advised. The prolan test of Dec. 24, 1933, showed less than 100 M.U.

CASE 9.—White, aged thirty-four. Formerly admitted here for pulmonary tuberculosis and tuberculosis of the gastrointestinal tract, as well as for heart trouble. No previous history of injury. In 1931 first noted swelling of right testicle. On admission here in May, 1933, a right orchidectomy was done. The histologic diagnosis was teratoma. Irradiation treatment given to postoperative scar, abdomen, mediastinum, right and left scapular regions. Prolan test of October, 1933, was negative.

CASE 10.—White, aged forty-five. No history of injury. Had a right orchidectomy at another hospital in January, 1933. Two months later he noted abdominal pains. Admitted here October, 1933, with diagnosis of abdominal tumor, carcinoma of lungs, nodes in left supraclavicular region. Prolan test showed 16,000 M.U. Irradiation therapy was attempted but the critical condition of the patient precluded the continuance of such treatment. Patient died a few days after admission. Autopsy showed extensive metastases to the abdomen, lungs, neck lymph glands, and to other lymph glands. The metastatic nodules were of the teratoma type.

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A NOTE ON FRIEND'S METHOD FOR THE ESTIMATION OF CHLORIDES*

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WHEN Herman Friend's method† for the direct titration of chlorides in blood appeared, it seemed for simplicity to leave nothing to be desired. However, the first estimation made with the new method (on a specimen of normal blood) was considerably higher than had been expected.

The silver nitrate solution was then titrated against a 0.5 per cent sodium chloride solution and was found to be of lower concentration than theory demanded. The source of such errors is of little import as compared with the consideration that they are prone to occur, and to affect the results of a series of determinations if not detected.

The factor 1.17 in Friend's equation had to be reduced, and his table was consequently rendered useless. It was then deemed best to make a solution of silver nitrate (2.906 gm. per liter) of which 1 c.c. would represent 1 mg. of sodium chloride. With this change, the table is not needed.

The modified procedure is as follows:

To 1 c.c. of plasma 3 c.c. of the aluminum hydroxide cream is added after diluting the plasma with 10 c.c. of distilled water. The volume is then made up to 25 c.c. with water. A drop of caprylic alcohol may be used to dispel the foam, thus increasing the accuracy of measurement. After mixing and letting stand for ten minutes, the mixture is filtered. To 20 c.c. of the filtrate 5 drops of 5 per cent potassium chromate is added, and the filtrate is titrated with the standard silver nitrate of which 1 c.c. represents 1 mg. of sodium chloride. The end-point is the change from yellow to brownish, and it should be the same tint as that reached in the standardization of the silver salt against the sodium salt. When the number of cubic centimeters of the silver nitrate solution used is multiplied by 125, the result expresses the chloride content in mg. NaCl per 100 c.c. of plasma.

The method is applicable to urine also. For this use it is even simpler, as preliminary treatment is usually unnecessary. One cubic centimeter of urine in approximately 25 c.c. of distilled water is titrated without filtering. The number of cubic centimeters required corresponds to the chloride content in gm. NaCl per liter.

It is desired specifically to avoid the implication that Friend's factor 1.17 is either theoretically or practically erroneous. The substitution of the standard solution (containing silver nitrate representing 1 mg. NaCl per c.c.) for the 0.02 normal solution is for convenience in calculation merely. The pub-

*From the Pathological Laboratory of the French Hospital.
Received for publication, March 13, 1934.
†Friend, H.: Estimation of Chlorides in Blood, J. Biol. Chem. 51: 115, 1922.

lication of the results of titrations of pure chloride solutions in this connection would be a work of supererogation; it would prove nothing except the accuracy or inaccuracy of the chemist doing the work. The comparison made by Friend of results with his own method and with the standard one of McLean and Van Slyke would be duplicated if the modified method were used by a chemist of equal competence, and under the same conditions, for this method is still essentially Friend's.

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THE DETERMINATION OF URINE CHLORIDES WITH MERCURIC NITRATE*

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THE chloride method of Votoček[1] using mercuric nitrate to titrate chloride solutions with sodium nitroprusside as an indicator was applied by Cavett and Holdridge[2] to the Folin-Wu filtrate in the determination of blood plasma chlorides. This method has proved very satisfactory and has been applied to the determination of urine chlorides.

METHOD

Five cubic centimeters of urine are placed in a 100 c.c. volumetric flask and diluted to volume. A 5 or 10 c.c. aliquot is taken for the titration using the same reagents and the exact procedure as given in the plasma chloride method for the titration of the Folin-Wu filtrate.†

Urines which contain protein give a turbidity with the mercuric ion which interferes with the end-point of the titration. Thus, with such urines, 5 or 10 c.c., depending on the chloride content, are placed in a 100 c.c. volumetric flask and 5 c.c. of 10 per cent *sulphosalicylic* acid are added. The flask is placed in a boiling water-bath for a few minutes, cooled, and diluted to volume. The contents of the flask are filtered through a dry filter. If the filtrate shows any turbidity it is returned to the filter until it comes through clear. The titration is then carried out on a 5 or 10 c.c. aliquot.

Comparison with the Volhard-Arnold silver nitrate method as given by Hawk and Bergeim[3] shows the error to lie within one per cent.

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*From the Laboratory of Physiological Chemistry of the University of Minnesota.
Received for publication, March 5, 1934.

†One cubic centimeter of the mercuric nitrate solution is equivalent to 1 mg. NaCl. The titration corrections given in Table I of the plasma chloride method are used.[2]

A NEW PROCEDURE FOR INTRODUCING SOLUTIONS DIRECTLY INTO THE SMALL INTESTINE OF EXPERIMENTAL ANIMALS*

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RECENTLY a technic was reported (Maddock, 1932) which permits ligation of the pylorus of animals from the exterior without the complications of general anesthesia. Utilization of this procedure enabled Maddock, Trimble, and Carey (1933) to demonstrate that the absorption of d-glucose from the stomach is either zero or negligibly small.

The present communication describes a procedure which permits the rôle of the small intestine in absorption to be studied separately from that of the other portions of the alimentary tract of the dog. The technic of this is as follows: Under ether anesthesia with aseptic precautions a midline incision is made from the tip of the xyphoid to a point a few centimeters above the umbilicus. The glass pyloric snare is then inserted as described previously (Maddock, 1932). The wound is closed with interrupted silk sutures.

A right rectus muscle-splitting incision is then made and the loop of the duodenum, distal to the lower pancreatic duct (Santorini) is brought to the outside. The edges of the abdominal wall are approximated under the duodenum by placing 1 or 2 sutures through both peritoneum and fascia, in a manner similar to that described by Mann (1921). This allows about 5 cm. of duodenum to lie on the anterior rectus sheath where it is readily accessible (Fig. 1). The skin is then closed over the gut with interrupted silk sutures. Dry dressings are placed over the wound and held in place by a scultetus binder.

The animals recover rapidly from this operation and are usually willing to consume a meal of bread and milk or ground meat by the following day. After from three to seven days they may be used for an absorption test as described below. Somewhat less than 10 per cent of the experimental animals fail to recover their appetites promptly. A few of these show elevation of blood nonprotein nitrogen. All dogs which refused food for several days or whose appetite was poor gave subnormal results in absorption tests, so that a normal appetite appears to be an important criterion of the success of the preliminary surgery.

On the day of the absorption test the skin sutures are removed. The snare is drawn sufficiently taut to close the pylorus. A hollow needle is then inserted into the lumen of the duodenum, and the fluid whose absorption is to be studied is infused through the needle. An alternative procedure which is also feasible, is to make a 5 mm. incision in the duodenum through which a soft rubber catheter is inserted into the lumen. A purse-string suture is then placed about the opening and tied snugly around the catheter to prevent leakage.

*From the Surgical Research Laboratory of the Boston City Hospital.
Received for publication, March 8, 1934.

At the end of the experiment the animals are quickly and painlessly sacrificed by injecting sodium amytal (100 mg. per kilo) intravenously. The intestine can then be removed and completely washed out in order to make a quantitative study of absorption from the intestine or of chemical changes which may have occurred during the experimental period.

RESULTS

In order to test the procedure several types of experiment have been performed. In these the absence of hyperglycemia has been used as a criterion of the normality of the animal during the absorption test.

The animals were given the preliminary surgical preparation just described. On the day of the absorption tests (3 or 5 days after the preliminary

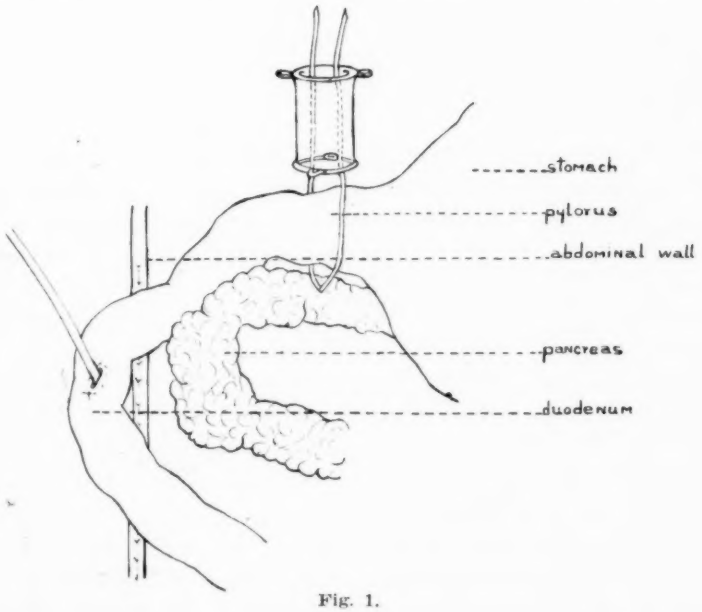

Fig. 1.

operation) the animals (fed eighteen hours previously) were brought into the laboratory and a sample of venous blood (I control) was obtained. The pylorus was then closed and the needle inserted into its duodenum. A second specimen of blood (II control) was procured.

Then with the aid of a Woodyatt pump fluids (water or sodium chloride) warmed to 37° C. were injected directly into the small intestine at constant rates. At fifteen-minute intervals specimens of blood were collected from the saphenous veins. The sugar content of these was determined by the ferrocyanide method of Folin and Malmros (1929).

The data obtained are recorded in Table I. These show that neither the manipulative procedures immediately preceding the injection nor the infusion of considerable volumes of fluids directly into the small intestine provoked a hyperglycemic response of any significant magnitude.

TABLE I

EXPERIMENT NUMBER	I	II	III	IV
Weight of dog, kg.	17.0	15.1	23.5	15.2
Substance ingested	H_2O	NaCl	NaCl	NaCl
Concentration per cent		0.9	2.5	2.7
Volume, c.c.	800	450	640	820
Blood Sugar Concentrations, Milligrams Per Cent, During Injection				
I Control	87	85	80	70
II Control	89	92	78	72
15 minutes	92	92	81	74
30 minutes	93	91	82	75
45 minutes	88	92	83	73
60 minutes	90	82	84	69
75 minutes	89	89	82	74
90 minutes	85	87	86	76
105 minutes	90	89	82	74
120 minutes	89	89	85	83
Corpuscle Volume, Per Cent				
Before injection	47, 48	43, 42	44, 45	52, 52
After injection	46, 46	40, 39	34, 33	52, 53

Leakage from Intestine into Stomach.—That the pyloric snare is able to prevent solutions passing backward into the stomach was shown in the following tests. D-glucose (concentrations 3-22 per cent, volumes 100-600 c.c.) was infused directly into the duodenum. After completion of the infusion (which consumed from one to three hours), the content of the stomach was collected and tested for the presence of reducing substances. In a series of 9 experiments the quantities found were respectively 22, 95, 29, 162, 9, 90, 135, 43, and 32 mg. In a previous communication Maddock, Trimble and Carey (1933) have shown that d-glucose is not absorbed directly from the stomach. Therefore the data just presented demonstrates that leakage from the intestine into the stomach need not occur when the procedure presented in the present communication is employed.

The technic described above provides a method for introducing a wide variety of substances into the intestine of an intact animal under conditions which avoid the necessity of general anesthesia or permanent fistulas.

The Rate of Absorption of Sodium Chloride.—Incidental to the main purpose of the experiments recorded there was opportunity for a few observations upon the rate at which sodium chloride is absorbed from the intestine. Computations based upon the data of Experiment III show that in this instance the total sodium chloride ingested was 16.0 gm. Of this 1.43 gm. was recovered from the intestine at the conclusion of the experiment. Therefore 14.57 gm. of sodium chloride had been absorbed during two hours. This occurred at the rate of 0.31 gm. per kilo of body weight per hour. In Experiment IV the sodium chloride infused totaled 22.14 gm. The recovery was 9.05 gm. and the absorption rate was 0.42 gm. In Experiment III dosage of sodium chloride was 0.68 gm. per kilo while in Experiment IV it was 1.45 gm. per kilo. In both experiments the concentration of the solution was substantially the same. These observations together with others not included here, present some indication that an increased dosage of sodium chloride produces an

increase in the rate at which this salt is absorbed from the intestine. This is in contrast with the observation of Trimble and Maddock (1934) that the rate of absorption of d-glucose from the intestine of the dog is not significantly altered by variations in its dosage.

This procedure was developed and the accompanying observations were made during the course of an investigation carried on in collaboration with Doctor Harry C. Trimble of the Department of Biological Chemistry, Harvard Medical School.

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A SIMPLIFIED TECHNIC FOR THE COLORIMETRIC DETERMINATION OF BLOOD CHOLESTEROL

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THE inherent technical difficulties in the methods of Bloor,[1] Sackett,[2] and Myers and Wardell[3] for the determination of blood cholesterol have made a simple colorimetric method highly desirable. In 1928 Cornell[4] described a colorimetric technic for the determination of blood and tissue cholesterol based upon the drop precipitation of blood in an alcohol-ether mixture at room temperature. At that time we compared the results obtained by this alcohol-ether extraction with those obtained by the older methods and were able to verify Cornell's statement that extraction by this method is complete.

In following the further directions of Cornell (consisting of washing of the precipitation flask and the precipitate with alcohol-ether, reducing the filtrate volume by distillation and the use of a part of the reduced filtrated for final evaporation) the method was found to be somewhat cumbersome and because of the several steps involved, subject to technical errors.

Separate examinations of the precipitate washings revealed the fact that they contained insufficient cholesterol to give a readable color development by the Burchard Leibermann reaction. We accordingly eliminated the washings and with them the necessity of reduction of the filtrate volume by distillation. We are now using an aliquot part of the original filtrate for the determination, a procedure entirely in accord with other blood chemical determinations in which the Folin-Wu filtrate is used.

*From the Department of Pathology, White Cross Hospital.
Received for publication, March 12, 1934.

In the use of the Burchard-Leibermann reaction for the maturation of color in the chloroform solutions of cholesterol, control of three variable factors is necessary if accurate colorimetric comparisons are to be obtained. Color develops slowly in cold solutions and rapidly in warm solutions; greater intensities of light also accelerate the color development. If conditions of temperature and light are standardized, time of development remains the only variable factor. If chloroform solutions of pure free cholesterol are used as the standard and exposed to the same physical conditions of temperature and light as the unknown samples, the development of color in both standard and unknown are presumably parallel and the exact time of maximum development of color at which colorimetric comparisons are to be made may vary within certain arbitrary limits without introducing clinical errors. If, however, the more convenient artificial standard is used for comparisons, the time factor must be constantly under control in order that colorimetric comparisons may be made at the exact point of maximum color development in the unknown solution. It is obvious that this time will vary with each determination, being influenced by the room temperatures, temperature of the solutions, and the various light conditions.

It is desirable that color maturation progress slowly. In order to obtain slow color development by the control of the variables of light and temperature Cornell directs that the containers be chilled in an ice bath, then placed at an arbitrary fixed distance from a 200 watt lamp in a dark room for forty-five minutes. In 1932 Mirsky and Bruger[5] and later Mirsky[6] directed that the containers be placed in a dark closet at room temperature for five minutes and then for another ten minutes in the refrigerator. It is obvious that the variabilities of room temperature in either procedure would influence the exact time set for comparison of the developed colors, but as these authors were using the natural standards the time factor was of minor importance.

In order to use the more convenient artificial standard we have modified our procedure for color maturation. We now develop color by the Burchard-Leibermann reaction at room temperature throughout and expose the unknown solution to the same light by which the subsequent color comparisons are to be made, in the cups of the artificially illuminated colorimeter itself. By this method repeated colorimetric comparisons are possible during the entire range of color maturation and fading and the exact point of maximum color development is not missed.

PROCEDURE

Into a 125 c.c. Erlenmeyer flask place exactly 24 c.c. of a two to one mixture of alcohol and ether. From a 1 c.c. volumetric pipette add drop by drop and with constant agitation 1 c.c. of whole oxalated or hepranized blood. Cork tightly and agitate for two minutes. Filter through a fat-free paper into a stoppered 50 c.c. wide mouth bottle.

Pipette 5 c.c. of this filtrate (an aliquot part) into a 50 c.c. Erlenmeyer flask and evaporate on the "low" heat of an electric hot plate until about one drop of solution remains. Remove at this point to prevent complete drying and overheating with possible discoloration of the residue. Add at once

5 c.c. of chloroform. Cork tightly and allow to stand until it cools to room temperature. The addition of the chloroform to the residue while hot assures complete solution of the cholesterol. When making a series of examinations, the flasks may stand stoppered at this point until it is convenient to develop the color.

When cool add 0.2 c.c. of concentrated sulphuric acid, stopper and cool to room temperature, then add 1 c.c. of acetic anhydride. Allow to stand for two minutes, transfer to the colorimeter cups and compare with the standard every minute until constant maximum readings are obtained, then for several minutes more until the color fades, through three successive readings.

The standard used is National Aniline Company's Naphthol Green B from which a 0.1 per cent aqueous stock solution is prepared. The stock is stable for several weeks but must be diluted fresh before using. If 1 c.c. of this stock is diluted with 19 c.c. of water the resulting color is equivalent to that produced by 0.0004 gm. of pure cholesterol dissolved in 5 c.c. of chloroform.

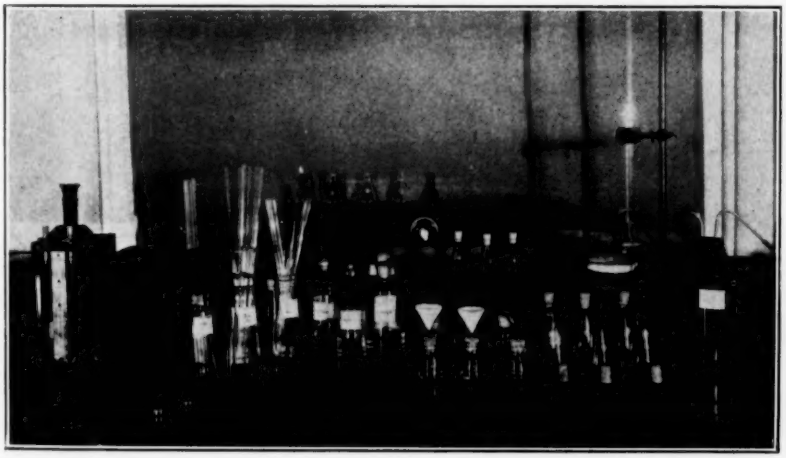

Fig. 1.—Apparatus for blood cholesterol determination, showing precipitation flasks, filtration bottles, evaporation flasks, hot plate, buret for alcohol-ether mixture and the artificially illuminated colorimeter used in these experiments.

Since the unknown cholesterol in the 5 c.c. of chloroform solution is further diluted by the addition of 0.2 c.c. of sulphuric acid and 1 c.c. of acetic anhydride it is necessary to add a further 1.2 c.c. of water to every 5 c.c. of the diluted standard. By setting the unknown at 20 on the colorimeter scale and reading the standard the calculations become: Standard × 10 = Mg. Cholesterol in 100 c.c. of whole blood.

The apparatus used by the author is shown in Fig. 1.

DISCUSSION

The practical accuracy of the above method is demonstrated by the following check determinations made on four different blood samples:

Sample 1	Determination 1	161 mg.
	Determination 2	160 mg.
	Determination 3	161 mg.
	Determination 4	160 mg.

Sample 2	Determination 1	240 mg.
	Determination 2	239 mg.
	Determination 3	241 mg.
	Determination 4	240 mg.
Sample 3	Determination 1	132 mg.
	Determination 2	133 mg.
	Determination 3	131 mg.
	Determination 4	132 mg.
Sample 4	Determination 1	115 mg.
	Determination 2	114 mg.
	Determination 3	114 mg.
	Determination 4	115 mg.

The variations in the above determinations are within the limits of error inherent in all colorimetric readings.

The control of the variables of time, light, and temperature by constant colorimetric observations of the maturating colors is illustrated by the following readings taken at random from the records of cholesterol determinations. These determinations were made on different days under different conditions of room temperature and represent blood from normal and pathologic cases.

Sample 1	3 minutes	125 mg.	
	4 minutes	135 mg.	
	5 minutes	150 mg.	
	6 minutes	155 mg.	Maximum in 6 minutes
	7 minutes	155 mg.	
	8 minutes	154 mg.	
	9 minutes	152 mg.	
	10 minutes	145 mg.	
Sample 2	3 minutes	88 mg.	
	4 minutes	93 mg.	
	5 minutes	95 mg.	
	6 minutes	100 mg.	
	7 minutes	105 mg.	
	8 minutes	110 mg.	
	10 minutes	122 mg.	
	11 minutes	130 mg.	
	12 minutes	131 mg.	Maximum in 12 minutes
	13 minutes	131 mg.	
	14 minutes	125 mg.	
	15 minutes	115 mg.	
	16 minutes	105 mg.	
Sample 3	3 minutes	40 mg.	
	4 minutes	52 mg.	
	5 minutes	80 mg.	
	6 minutes	116 mg.	
	7 minutes	140 mg.	
	8 minutes	164 mg.	Maximum in 10 minutes
	10 minutes	165 mg.	
	11 minutes	165 mg.	
	12 minutes	163 mg.	
	13 minutes	162 mg.	

The effect of temperature, as well as the practical accuracy of the method, is illustrated by the following colorimetric readings of three separate determinations on a single sample of blood containing an abnormally high amount of cholesterol. In these determinations the acetic anhydride was added to the warm, partially cooled and room temperature chloroform solutions of the cholesterol residue after the addition of the sulphuric acid.

WARM		PARTIALLY COOLED		ROOM TEMPERATURE	
2 minutes	60	2 minutes	40	2 minutes	30
3 minutes	82	3 minutes	74	3 minutes	42
4 minutes	100	4 minutes	100	4 minutes	70
5 minutes	184	5 minutes	170	5 minutes	96
6 minutes	210	6 minutes	192	6 minutes	100
7 minutes	260	7 minutes	222	7 minutes	116
8 minutes	272	8 minutes	240	8 minutes	140
9 minutes	290	9 minutes	253	9 minutes	163
10 minutes	298	10 minutes	273	10 minutes	190
11 minutes	300	11 minutes	287	11 minutes	220
12 minutes	300	12 minutes	298	12 minutes	252
13 minutes	299	13 minutes	300	13 minutes	268
14 minutes	299	14 minutes	300	14 minutes	279
15 minutes	296	15 minutes	301	15 minutes	288
16 minutes	290	16 minutes	300	16 minutes	296
		17 minutes	294	17 minutes	299
Maximum in 11 min.		18 minutes	290	18 minutes	301
				19 minutes	301
		Maximum in 15 min.		20 minutes	302
				21 minutes	301
				22 minutes	300
				23 minutes	297
				Maximum in 20 min.	

SUMMARY AND CONCLUSIONS

1. A simplified and accurate technic for the determination of blood cholesterol is described. We do not present this technic as entirely new but only as a simplified method which has proved highly satisfactory.

2. By the elimination of unnecessary washings and reduction of excess filtrate volume by distillation, time is saved and accuracy increased. The use of an aliquot portion of the filtrate is quantitatively accurate.

3. The use of naphthol green B as the artificial standard is entirely satisfactory and much less difficult than the preparation of chloroform solutions of pure free cholesterol.

4. By developing color at room temperatures in the artificial light of the colorimeter a series of comparisons can be made throughout color maturation and absolute control of the variable factor of time is attained.

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PROTEIN ANALYSES IN CEREBROSPINAL FLUID*

A Comparative Study of Methods

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SINCE the introduction by Denis and Ayer[1] of a rapid and simple method for the estimation of the protein content of cerebrospinal fluid, this particular analysis has acquired considerable diagnostic import. It is generally accepted by all clinical analysts, as far as our inquiries have extended, that the Denis and Ayer method is entirely satisfactory for this measurement, being both rapid and, within certain limits, exact.

In a later communication, in which the original Denis and Ayer technic is slightly modified, Ayer, Dailey and Fremont-Smith[2] state that "The exact magnitude of the error inherent in the method has not been determined, since there is no more accurate method to use as a check on individual samples of normal fluid." They present comparative figures between the modified Denis and Ayer method, and macro- and micro-Kjeldahl determinations in which the protein nitrogen is estimated *by difference;* that is by subtraction of the non-protein nitrogen from the total nitrogen. As they are careful to point out, such an indirect method permits a possibility of rather large accumulation of error. The actual percentage error in these comparative figures ranges from 2.6 per cent to 17.6 per cent, with a mean value of 8.6 per cent, which it should be remembered represents the algebraic sum of the errors of both analyses.

In view of the wide clinical use of the Denis and Ayer method, and the admitted question as to its exactness, it has seemed advisable to attempt to check the results obtained with the Denis and Ayer method compared with two other methods, both based upon entirely different analytical principles. One of these is a micro-Kjeldahl procedure, in which the protein nitrogen is determined by *direct* digestion of the precipitated protein. This method, already published by Walker and Sleeper,[3] has been shown by them to agree with macro-Kjeldahl determinations on dilute egg albumen solutions with a maximum error of 3 per cent. The other is a colorimetric method, dependent on the production of a color by reducing a protein-phosphomolybdic acid precipitate.

EXPERIMENTAL

Denis and Ayer Method.—It was carried out according to the modified procedure of Ayer, Dailey and Fremont-Smith,[2] and using a Bausch and Lomb biologic colorimeter fitted with the special small cups and plungers as recommended in that same communication.

Micro-Kjeldahl Method.—It was carried out according to the procedure of Walker and Sleeper,[3] and using an ordinary biologic colorimeter.

*From the Evans Memorial (Massachusetts Memorial Hospital) and the Fifth Medical Service, Boston City Hospital.

Received for publication, March 19, 1934.

Colorimetric Method.—To 1 c.c. of fluid in a small test tube (about 5 c.c. capacity) is added 1 c.c. of 1 per cent phosphomolybdic acid in normal sulphuric acid. After mixing and standing for fifteen minutes, this is centrifuged, the supernatant liquid decanted as completely as possible and discarded. The precipitate is resuspended in water, again centrifuged and the supernatant liquid discarded. (This washing is essential to remove excess of unprecipitated phosphomolybdic acid.) To the washed precipitate is now added 1 c.c. of fifth normal sulphuric acid, 1 c.c. of 2 per cent hydroquinone, and after shaking, 1 c.c. of the carbonate-sulphite solution used in the Bell and Doisy[4] method for phosphates (15 per cent sodium carbonate and 3 per cent sodium sulphite). The color is read in the colorimeter fitted with small cups against a standard similar to that used in the Denis and Ayer method, treated in the same manner as the unknown fluid. The colorimetric reading should be made promptly, as the color has a tendency to fade rather rapidly.

ANALYTICAL RESULTS

It is first necessary to show that the new colorimetric method yields results comparable to the macro-Kjeldahl, which still remains our standard of protein determinations. Table I shows the results obtained with dilutions of a stock protein solution, made up and standardized according to the procedure of Denis and Ayer in preparing their protein standard.

TABLE I

MG. PROTEIN PER 100 C.C. (MACRO-KJELDAHL)	MG. PROTEIN PER 100 C.C. (COLORIMETRIC)	PERCENTAGE ERROR
159.0	155.0	2.5%
119.0	121.0	1.7%
79.5	79.0	0.6%
59.5	59.0	0.8%
23.8	22.3	6.3%
19.8	18.9	4.5%

Inspection of Table I shows that with moderately increased amounts of protein this method gives results which are accurate within the limits of personal error in colorimetric reading. In the lower ranges the color is more difficult to apprehend, and the error is somewhat, although not prohibitively, greater.

The three methods were next compared on three samples of pooled cerebrospinal fluid, the results of which are shown in Table II.

TABLE II

POOLED CEREBROSPINAL FLUID

Mg. Protein per 100 c.c. Fluid

BY MICRO-KJELDAHL METHOD	BY DENIS AND AYER METHOD	BY COLORIMETRIC METHOD
33.5	32.5	33.0
32.4	30.5	32.0
22.1	20.5	21.7
Percentage Recovery (Assuming Micro-Kjeldahl as 100 Per Cent)		
100	97	98.5
100	94	99.0
100	93	98.0

Inspection of Table II shows that on the same fluid the three methods give essentially the same results. Both the Denis and Ayer method and the new colorimetric method yield results slightly, but hardly significantly lower than the micro-Kjeldahl. By its simplicity, the Denis and Ayer method recommends itself as the most satisfactory for clinical use. The micro-Kjeldahl procedure remains, we believe, the standard procedure for exact determinations. The colorimetric method may perhaps find a use as a rapid check method, but we feel that it has already served its most important purpose in proving, we trust conclusively, that the Denis and Ayer method is valid and dependable.

SUMMARY

Three methods for the determination of small amounts of protein, all found accurate when tried with dilute protein solutions, have been applied to samples of pooled spinal fluid, and have yielded similar results. The method of Denis and Ayer being the simplest, is recommended for use in clinical spinal fluid protein estimations.

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FURTHER OBSERVATIONS ON THE COLLOIDAL CARBON FLOCCULATION TEST IN SPINAL FLUID*

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IN AN earlier publication[1] the method of performing a colloidal carbon flocculation test in spinal fluid was described in detail. Since that publication the work on the method has been continued and although the fundamental principles have remained as before, several minor alterations have been introduced. It is the purpose of this paper to describe them and the results obtained.

ALTERATIONS OF THE TEST

In the course of the work upon the test, it was found that a set of six tubes was unnecessary except in cases of meningitis in which the fluid was not sterile. Four tubes in the set were sufficient for all other spinal fluids. Into each tube was placed 1 c.c. of distilled water. In the first tube of the set was placed 1 c.c. of spinal fluid. It was thoroughly mixed and 1 c.c. of this mixture was

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Received for publication, April 6, 1934.

transferred to the second tube of the set. This process was repeated through the four tubes, the last 1 c.c. being discarded. Into each of the four tubes 0.1 c.c. of 0.1 per cent oxalic acid solution was measured. Each tube was inverted several times to mix. Then into each tube was added 0.4 c.c. of 1 per cent suspension of colloidal carbon. Each tube was again inverted several times to mix.

Dual controls were set up with each spinal fluid, one with oxalic acid and one without oxalic acid in order to establish comparative extremes.

The set was kept at room temperature and read twelve hours later as has been described previously.

RESULTS

This test has been performed upon 552 spinal fluids. Of these the distribution was:

General paralysis	50 cases	Epilepsy	26 cases
Tabeo-paresis	22	Senile deterioration	18
Cerebrospinal syphilis	17	Psychoneurosis	18
Meningovascular syphilis	12	Psychopathic personality	17
Schizophrenia	60	Organic brain disease	16
Cerebral arteriosclerosis with psychosis	50	Posttraumatic constitution Without psychosis	14 12
Manic depressive psychosis	48	Hydrocephalus	11
Alcoholic psychosis	42	Multiple sclerosis	11
Mental deficiency	36	Cerebral hemorrhage	9
Senile psychosis	33	Diabetes insipidus	3
Toxic psychosis	27		
		Total	552 cases

In these cases the carbon test was positive only in the spinal fluid of the cases of neurosyphilis. In all others it was negative. No relationship could be established between the test and the spinal fluid protein, proteose, sugar, chlorides, pH, or cell count.

DISCUSSION

Colloidal carbon when in suspension carries a negative electrical charge. Oxalic acid in solution carries a positive electrical charge. When these two are mixed the negative charge on the carbon particle is neutralized, the particles due to decreased surface tension aggregate and flocculate out, leaving a solution whose clearness depends upon the degree of flocculation.

When oxalic acid is added to normal spinal fluid, the positive electrical charge is absorbed completely by the existing free negatively charged particles in the spinal fluid so that when the colloidal carbon is added its charge remains unchanged and the carbon remains in suspension; the mixture remaining black.

But in neurosyphilitic spinal fluid there do not exist sufficient "free" negative electrical charges to neutralize the positive electrical charges of the oxalic acid, so that when the oxalic acid is added its positive electrical charges to a large extent remain unchanged. When colloidal carbon is added to this mixture, its free negative electrical charges are neutralized by the positive electrical charges of the oxalic acid, and the carbon flocculates out. The protective qualities of the protein in the spinal fluid are made inert by the addition of distilled water to the mixture.

Another way to explain this phenomenon more easily is thus:

In the test an organic sol, an inorganic sol, and an electrolyte are used. When the organic sol is mixed with the inorganic sol of the same charge of particles, the organic sol protects the particles of the inorganic sol from flocculation by electrolytes. The organic sol is the spinal fluid; the inorganic sol is the colloidal carbon; and the electrolyte is the oxalic acid. In neurosyphilitic spinal fluids the activity of this protective organic sol is decreased in dilutions corresponding to Tubes 1, 2, and 3.

SUMMARY

1. The spinal fluids of 552 individuals have been examined by means of the colloidal carbon flocculation test.

2. Flocculation occurs only in the spinal fluids of neurosyphilis.

3. The possible mechanism of the test is described.

REFERENCE

1. Schube, P. G., and Harms, H. E.: A Colloidal Carbon Flocculation Test in Spinal Fluid. Preliminary Report, J. Lab. & Clin. Med. 18: 65, 1932.

A MICROMETHOD FOR THE DETERMINATION OF BLOOD NONPROTEIN NITROGEN*

H. Irving, L.T., and J. C. Forbes, M.A., Ph.D., Richmond, Va.

SINCE it is sometimes very convenient to have a nonprotein-nitrogen method for which blood may be readily obtained either from the finger or ear lobe, we have modified a micromethod in use in our laboratory for a number of years for that purpose. These methods are modifications of the well-known Folin and Wu method, but seem to offer several advantages over the same. Not only is the amount of blood filtrate required and the time needed for digestion much less, but also the tendency for precipitation during digestion is greatly reduced. This is the case if the digestion, especially the latter part, is carried on slowly. In some cases, if the nitrogen content is high it is almost impossible to prevent precipitation during digestion. Consequently in these cases, the determination should be repeated using a lesser amount of filtrate.

The procedure used for our regular method is essentially the same as Folin and Wu's method, except that one-fifth the amount of each solution is used and the tubes are graduated at the 10 c.c. instead of the 50 c.c. mark. The procedure is as follows: 1 c.c. of blood filtrate is transferred to a Pyrex test tube, 150 by 15 mm., graduated at 10 c.c., 0.2 c.c. of Folin and Wu digestion mixture added, and digestion carefully carried out in the usual manner. Distilled water is then added to the 10 c.c. mark, the solution mixed, and 3.5 c.c. of Koch's

*From the Departments of Biochemistry and Medicine, Medical College of Virginia.
Received for publication, April 14, 1934.

Nessler solution added. A standard is prepared at the same time, using 2 c.c. of an ammonium sulphate solution (1 c.c. = 0.02 mg. N.), 0.2 c.c. of digestion mixture added, diluted to 10 c.c. and 3.5 c.c. of Nessler solution added.

TABLE I

COMPARATIVE NONPROTEIN-NITROGEN DETERMINATION BY SEVERAL METHODS. VALUES IN MG. PER 100 C.C. OF BLOOD

DETERMINATION	FOLIN & WU'S METHOD	MICRO-METHOD	FINGER METHOD
1	27.2	28.6	29.2
2	38.8	37.8	38.2
3	35.2	35.0	34.8
4	30.8	30.4	31.0
5	37.0	36.0	37.8
6	35.8	34.6	34.0
7	38.4	38.0	38.6
8	31.0	29.8	28.6
9	34.0	34.0	33.8
10	----	38.0	40.0
11	----	36.0	36.0
12	----	24.0	24.0
13	----	31.8	31.6
14	----	18.2	18.0
15	----	31.4	30.6
16	----	33.4	34.0
17	----	37.8	37.8
18	----	28.4	30.0
19	----	40.0	40.0
20	----	39.0	39.0
21	----	36.0	35.8

In the first 14 the same blood was used for the three determinations, the same filtrate being used for the Folin and Wu and the micromethod. For the remainder, blood was taken by venapuncture in one case and from the finger in the other.

The finger tip method is essentially the same as above, 0.2 c.c. of blood is accurately measured in a special pipette and added to 3.4 c.c. of distilled water in a 15 c.c. centrifuge tube, the pipette being rinsed out several times with the diluting fluid. Two-tenths cubic centimeters of a 10 per cent sodium tungstate solution is then added, followed by 0.2 c.c. of $\frac{2}{3}$ N. sulphuric acid. After mixing and standing for several minutes, the tube is centrifuged at a moderately high rate of speed. Two cubic centimeters of the supernatant fluid is carefully digested with 0.2 c.c. of digestion mixture and analyzed as described above. If desired, the supernatant liquid may be filtered through a small filter paper after centrifuging, but this is as a rule unnecessary.

SUMMARY

A micromethod for the determination of blood nonprotein-nitrogen has been described, which requires only 0.2 c.c. of blood and is consequently suitable for finger tip or ear lobe blood.

NOTE: Suitable 0.2 c.c. pipettes may be obtained from A. S. Aloe Co., St. Louis.

A NEW METHOD FOR THE DETERMINATION OF THE BLOOD CELL VOLUME*

SIMON JAY MASON, CINCINNATI, OHIO

IN THE study of red blood corpuscles, a simple method was developed for the determination of their volume, which has an accuracy of ±0.2 per cent and which requires only a few drops of blood. The instrument employed is a bulb and calibrated capillary stem made of Pyrex which makes use of the hematocrit principle. It is 12 cm. in length and 1 cm. in diameter. The horizontal and

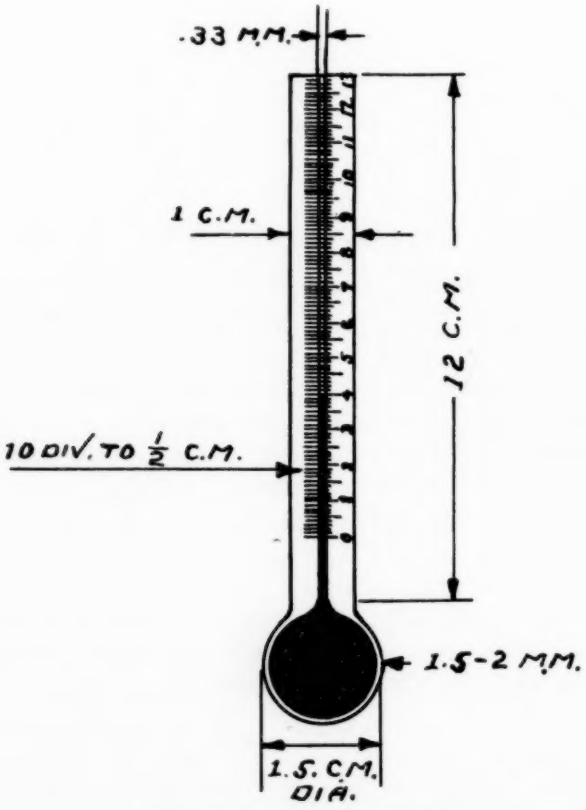

Fig. 1.—Bulb and calibrated tube.

vertical diameters of the bulb are approximately 1.5 cm.; and the thickness of its wall should not be less than 1.5 mm. The bore is 0.33 mm. and accurately calibrated. The outside markings are spaced to give ten to every half centimeter (Fig. 1).

*From the Kettering Laboratory of Applied Physiology, in the University of Cincinnati. Received for publication, April 9, 1934.

The bulb, filled with mercury, is heated in a small yellow gas flame and when the rising mercury column reaches the level of the tube, sufficient heparinized blood is placed upon the orifice and the bulb immersed in cold water. The contracting mercury draws after it a column of blood which fills the capillary. Excess blood is wiped off and the instrument is placed in a centrifuge cup; in the latter it is centered with a flanged cork, balanced against a cup containing the required amount of sand, and centrifugalized to constant volume. One half hour at 3,800 r.p.m. is sufficient for the purpose. The height of the entire column of the sample and that of the corpuscles is read from the calibration on the outside of the tube (greater accuracy being secured with the aid of a lens), and the percentage of red blood cells per hundred cubic centimeters is calculated.

To remove the blood, the bulb is heated in a blue Bunsen flame until all the mercury is driven out. The stem must not be heated. This leaves the tube

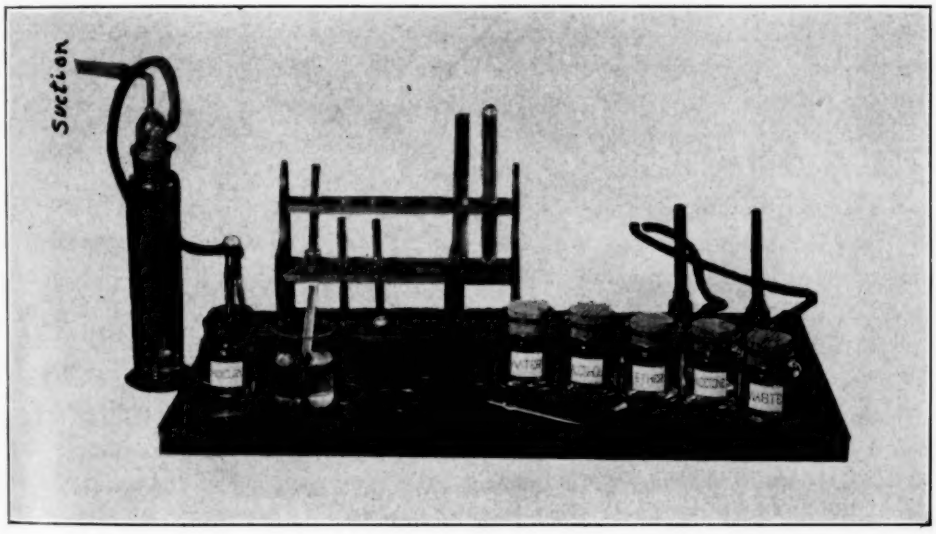

Fig. 2.—Apparatus.

clean and ready for refilling. The instrument can be used several times before any other cleaning is necessary. When this is needed, the empty bulb is heated slightly and inverted into a test tube containing concentrated sulphuric acid. The acid is then drawn up into the cooling bulb. After a few hours the acid may be removed by renewed heating. The outside of the tube is washed with water and inserted, stem down, through one opening of a two-hole rubber stopper; the other opening is occupied by a piece of glass tubing connecting to a strong water jet exhauster. The rubber stopper is now inserted successively into the neck of each of a series of jars containing, respectively, water, alcohol, ether, acetone; and the suction is so manipulated, together with turning the bottle upside down and righting it, that the bulb and tube are alternately filled with the desired cleaning material and emptied, repeatedly. The instrument is filled with mercury in the same way, the tube and jar being inverted and sustained by a rack. When the tube has been partially filled, the mercury is driven out by heating in

order to completely remove traces of acetone, the presence of which interferes with subsequent control of the mercury column. The bulb and tube are then refilled with mercury, centrifugalized for a minute or two, and the instrument is ready for use. The mercury column should reach about 2 cm. above the bulb. The equipment employed is illustrated in Fig. 2. (Precaution must be taken against the inhalation of vaporized mercury. It is advisable when emptying the bulb to have a jar into which the instrument, inserted in the rubber stopper, is placed, with suction going. The vapors of the mercury are thereby drawn off and condensed. The cleaning of the bulb should be carried out under a well-ventilated hood. The jars and the gas burners should be placed in a low rimmed pan so as to prevent the scattering of the mercury.)

Blood is obtained by drawing it from a freely bleeding finger or ear into a blood cell diluting pipette.* Before the blood is drawn, a 2 cm. column of 2 per cent solution of heparin is taken into the pipette stem, after which the pipette is filled to one-quarter or one-half of its volume. The slight dilution introduces only an inconsequential error. The pipette is gently shaken for thorough mixing with the heparin and the first drop of its contents discarded. The determination can be delayed for a while, but better results are obtained by carrying out the determination immediately.

A tenaculum forceps may be used to hold the instrument when the bulb is placed in the blue Bunsen flame for cleaning. When heating for the mercury rise, prior to the introduction of a blood sample, it is held by hand. Care must be taken not to immerse the bulb in water after heating in the blue flame, but breakage does not occur from immersion in water after heating in the yellow flame for a period sufficient to elevate the mercury column to the top of the tube.

During the centrifugation the cover of the centrifuge should be left open, in order to maintain air circulation to prevent an appreciable rise in temperature in the instrument and the sample above that of the room. For the same reason the bulb of the instrument must not be handled. The height of the blood column should be read immediately after centrifugation.

The following are the results (Table I) obtained when determinations were made on two samples of rabbit blood taken simultaneously from the same animal, employing two separate instruments.

TABLE I

RABBIT	% CELLS	RABBIT	% CELLS
13	35.9	38	27.8
	35.9		27.4
53	35.74	95	43.63
	35.3		43.85
55	33.64	3	39.1
	33.8		38.7
225	33.7	109	40.3
	33.3		40.7

*A number of short stemmed pipettes may be blown from small bored soda glass tubing, and the requisite amount of the 2 per cent heparin solution may be dried on the inside of these pipettes.

The following are the results (Table II) of a series of determinations made successively on the same animal, using separate instruments.

TABLE II

RABBIT	% CELLS	TIME	RABBIT	% CELLS	TIME
41	35.6	0 min.	438	35.6	0 min.
	35.2	20 min.		35.7	15 min.
	35.0	1 hr. 20 min.	59	33.5	0 min.
	35.3	2 hr. 20 min.		33.8	5 min.
	35.3	3 hr. 20 min.		33.9	25 min.
	35.0	4 hr. 20 min.		33.6	45 min.
83	39.7	0 min.		33.0	1 hr. 0 min.
	39.3	5 min.			
	39.6	20 min.	113	39.6	0 min.
	39.1	40 min.		39.8	35 min.
	39.1	1 hr. 0 min.		40.1	50 min.
	39.4	1 hr. 35 min.		40.0	1 hr. 20 min.
112	40.9	0 min.	57	39.1	0 min.
	40.8	20 min.		39.2	37 min.

SUMMARY

A modification of the hematocrit method for the determination of blood cell volume has been devised, which has an accuracy of ±0.2 per cent, and which requires only a few drops of blood.

SIMPLE APPARATUS FOR KEEPING CITRATED BLOOD WARM DURING INJECTION*

LEONARD HEDDICK, WHITTIER, CALIF.

THE simple and inexpensive apparatus here illustrated can be easily made out of material found in any laboratory. Citrated blood or other intravenous solutions can be readily kept warm by simply filling up the outer jar with warm water. As the temperature cools it can be drained out from the outlet provided in the bottom of the jar and more warm water added. The rate of flow of the blood is clearly visible, being greatly magnified. The apparatus is readily portable, does not need to be sterilized and can be used wherever warm water is available, as nothing else is required.

The warming chamber consists of an ordinary glass battery or museum specimen jar seven inches in diameter and seven inches deep. In the center of the bottom of the jar a circular hole is cut 1½ inches in diameter. A similar hole, 1 inch in diameter, is also cut out near the side. In this smaller hole a rubber stopper is fitted, through which is thrust a piece of glass tubing. A small piece of rubber tubing with pinch cock attached is fastened on the lower end to serve as the outlet for the cooled water.

*From the Murphy Memorial Hospital.
Received for publication, April 18, 1934.

A No. 8 rubber stopper and rubber tubing are slipped on to the lower end of a long tipped Kelly infusion jar. This part of the apparatus is kept wrapped and sterilized. When assembled the infusion jar will rest firmly upright upon the rubber stopper and require no additional support.

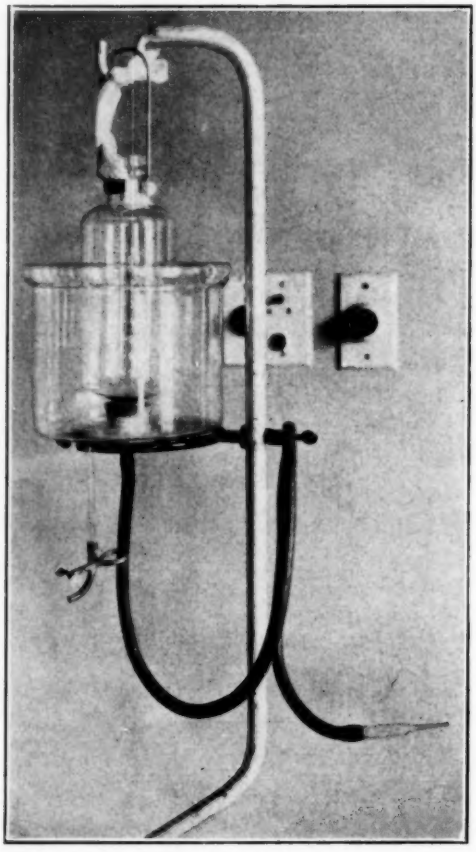

Fig. 1.

A small quantity of saline is used to start the injection, the apparatus is set on the iron ring holder, and the blood to be injected poured over the saline. The warming jar is then filled with warm water of the desired temperature.

The support on the standard is an iron ring with a one-inch segment cut out to allow the tubing to hang straight down.

This simple warming apparatus has been in use by the attending physicians of the above hospital for the past five years and has proved entirely satisfactory.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

INFLAMMATION: Cytological Picture of an Inflammatory Exudate in Relation to Its Hydrogen Ion Concentration, Menkin, V. Am. J. Path. **10:** 193, 1934.

A pleural inflammatory exudate, in the majority of instances, develops a rise in its hydrogen ion concentration concomitantly with the progress of the inflammatory reaction.

When the pH of the exudate is alkaline the percentage of polymorphonuclears at the site of inflammation exceeds that of the mononuclear phagocytic cells.

When the pH of the exudate is approximately neutral the percentage of polymorphonuclear cells tends to approach that of the mononuclear phagocytes.

When the pH of the exudate is definitely acid large numbers of polymorphonuclear cells are found degenerated. The percentage of relatively normal appearing polymorphonuclear leucocytes is found considerably lower than that of the mononuclear phagocytes.

In some cases the pH of the exudate remains alkaline throughout the period of an acute pleural inflammation. In these instances the percentage of polymorphonuclears invariably exceeds that of the mononuclears.

By measuring the hydrogen ion concentration of an inflammatory exudate the character of the cytologic picture can be predicted with a fair degree of certainty. Likewise the converse follows.

Evidence has been obtained to show that the development of a local acidosis in an area of inflammation precedes at times the changes occurring in the differential leucocyte formula of the exudate. In such cases, however, the cytologic changes ultimately follow the development of the acid reaction.

The observations reported suggest that the differential leucocyte formula in an area of acute inflammation is a function of the hydrogen ion concentration of the exudate. The cytologic picture in an inflamed area seems to be conditioned by the pH of the exudate surrounding the injured tissue. The present study indicates that the developing local acidosis as the inflammatory reaction progresses can adequately account for the shift in infiltration from polymorphonuclear leucocytes to mononuclear phagocytes at the site of inflammation.

DIPHTHERIA TOXOID, Evaluation of, and Methods of Immunization, Monroe, J. D., and Volk, V. K. Am. J. Pub. Health **24:** 342, 1934.

After considerable experience with toxin-antitoxin and toxoid, the authors are of the opinion that toxoid is preferable because it produces a higher degree of immunity in a shorter time. As a result of a previous study of 1,282 children, four injections of toxin-antitoxin gave 74 per cent of Schick negative children at the end of one year, while two injections of toxoid with alum gave 88.8 per cent Schick negative at the end of two months and 94.3 per cent at the end of one year.

Toxoid of 7½ to 10 antigenic potency was preferable to the preparation of concentrated toxoid used by the authors.

Toxoid with the addition of 0.2 per cent of alum is a preferable preparation because it produces a higher degree of immunity as determined by the Schick test in a shorter time than any other used in this study.

It appears that three 1 c.c. injections of toxoid with alum would be ideal, yet for practical application two injections are sufficient.

A 1 c.c. dose produces a slightly higher percentage of reactions than a ½ c.c. dose but produces a higher degree of immunity.

The slightly higher frequency of local reactions from toxoid with alum should not be an objection because the reaction is mild and short. Moderate general reactions occurred in 7 per cent of cases.

In order to reduce the number of visits to a minimum, injection of toxoid and the Schick test at the same visit is suggested for children over five years of age whenever there is no history of previous immunization.

GRANULOPENIA, Following the Use of Barbiturates and Amydopyrine, Randall, C. L. J. A. M. A. **102**: 1137, 1934.

Granulopenia should not be regarded as a primary type of blood dyscrasia until all possible etiologic factors have been eliminated.

An apparent increase in the occurrence of granulopenia may be related to the widespread use of drugs containing a benzene derivative.

A woman, aged twenty-five, developed an acute and alarming leucopenia following the use of barbiturates and amidopyrine for the relief of a simple headache.

The benzene chain contained in both drugs is possibly responsible for the severe reaction and the disappearance of the granulocytes from the blood.

A number of similar cases have been reported recently.

A difference may be expected in the reaction to benzene quickly absorbed from the alimentary tract, as compared to the type of benzene poisoning that may result from the prolonged, slow absorption that occurs in industrial exposure.

Individuals are known to vary greatly in their susceptibility to benzene. Granulopenia following the use of barbiturates and amidopyrine may therefore occur only in those individuals who are unusually susceptible to the benzene chain.

ANTIGEN, Note on Acetone Insoluble Lipoids in Relation to the Wassermann Reaction, Kolmer, J. A., and Richter, C. E. Am. J. Clin. Path. **4**: 235, 1934.

It is possible to increase the specific antigenic sensitiveness of alcoholic extracts of beef heart by increasing the amounts of alcohol soluble but acetone insoluble lipoids.

Such extracts sensitized with no more than 0.2 per cent cholesterol possess a very high degree of antigenic sensitiveness with no increase of nonspecific or anticomplementary properties.

Antigens of this kind permit the use of larger amounts in conducting the Wassermann test with an increase of specific sensitiveness for syphilis antibody.

BLOOD SEDIMENTATION, in Tuberculous Children, Reilly, W. A. Am. Rev. Tubere. 29: 220, 1934.

This study comprised 104 tests carried out on 36 tuberculous girls between the ages of eight months and fourteen years, from October, 1930, to March, 1931. Total white blood cell counts on fixed smears with Wright's stain and on supervitally stained smears (the latter for observing monocytes) were done at times for correlation and comparison.

The hilar lesion in its active stages, presumably in the hilum lymph nodes, was generally accompanied by a shortened sedimentation time. This was true in 22 of 23 such patients. The most rapid times, however, occurred in the blood of those children with more active lesions than are usually found in the hilar lymph nodes. It was thus when cavitation of the lung (2 patients) or miliary tuberculosis existed. Almost as rapid times were observed in patients with other disseminations, such as developing Pott's disease (2 patients) and the so-called allergic phenomena, phlyctenular keratoconjunctivitis (2 patients) and pleural effusions (2 patients). Occasionally, however, almost as short sedimentation times were found in the blood of children with hilar lesions.

Of the 33 patients considered clinically active, this was confirmed in 31 patients by a shorter sedimentation time. The remaining two patients had normal or longer times.

Compared or correlated with other laboratory tests for tuberculous activity, the sedimentation time indicated pathological effects much more frequently. Taking 6,000 to 12,000 as the normal range of total white blood cells, there were only rare increases in these children, whereas the Linzenmeier test always gave a rapid sedimentation time.

It is maintained that monocytes frequently increase in the blood of patients with tuberculous activity (3). The sedimentation time shows a consistent shortening. In 20 comparisons, the ratio of monocytes to lymphocytes did not indicate activity, whereas the sedimentation time was rapid in all 20 of these. Only once in 40 comparisons made on 23 patients did the ratio indicate activity and the sedimentation time fail to do so.

The Linzenmeier red blood cell sedimentation time done on 36 tuberculous girls 104 times was found to be a more reliable procedure in the prognosis than in the diagnosis of tuberculous activity. It is also of presumptive aid in diagnosing activity. The author believes it has an advantage over the usual signs, symptoms, and laboratory procedures in determining activity.

TULAREMIA, The Isolation from the Rocky Mountain Wood Tick of Strains of B. Tularense, etc., Davis, G. E., Philip, C. B., and Parker, R. R. Am. J. Pub. Health **19**: 449, 1934.

Three strains of *B. tularense* of low virulence for rabbits and guinea pigs have been isolated from *Dermacentor andersoni*. This is in marked contrast to numerous other isolations from this tick.

The occurrence in nature of strains of *B. tularense* of different degrees of virulence is indicated.

When making tests to determine the relative virulence of strains, it is believed that the use of both guinea pigs and domestic rabbits will prove advantageous.

ENTERITIS, Acute, in Infants and Young Children: Clinical Study in Forty-Six Patients: Necropsy Studies, Cooper, M. L., Keller, H. M., and Johnson, B. Am. J. Dis. Child. **47**: 596, 1934.

Five of the children were studied at necropsy. All showed gross and microscopic pathologic changes in the gastroenteric tract. Cultures of intestinal contents at various levels of the intestinal tract were made in the usual manner to avoid contamination; i.e., a sterile cotton swab was inserted through an incision in the intestinal wall made with sterile scissors in an area sterilized by application of a hot spatula. Postmortem cultures were made immediately after obtaining the material. This material was cultured and studied in the way described for cultures of stools.

Streptococci *(Streptococcus micro-apoikia)* were isolated from the contents of the involved intestine in four of these five patients and from the blood stream in the fifth. Four of these 5 cultures of streptococci were used for injection into animals, and all 4 cultures produced the characteristic pathologic changes in the animals' intestinal tract and were subsequently recovered from the animals.

A brief summary of the necropsy studies shows that of five patients studied at necropsy all had gross and microscopic pathologic changes in the intestinal tract and that *Streptococcus micro-apoikia enteritidis* was isolated from the intestinal tracts of 4 patients and from the blood of the fifth. Of 6 monkeys which received injections of this streptococcus 5 died, and these showed pathologic changes in the intestinal tract; streptococci were recovered from the intestinal tract of each. Ninety rabbits were given injections and 70 per cent of these died. Ninety-two per cent of these animals studied at necropsy showed characteristic pathologic changes in the intestinal tract, and cultures of the contents of the intestinal tract of animals at the time of necropsy showed the presence of *Streptococcus micro-apoikia enteritidis* in 80 per cent.

Streptococcus micro-apoikia was isolated from 55 per cent of 51 stools from 46 patients. The intestinal tracts of 5 patients cultured at necropsy contained these streptococci.

No streptococci similar to *Streptococcus micro-apoikia* were recovered in the control experiments in which seventy specimens of stools from 49 patients who had no intestinal infection were cultured.

Rabbits which received injections of the original mixed culture of stools or intestinal contents died; typical lesions were found in their intestinal tracts, and from these areas *Streptococcus micro-apoikia enteritidis* were recovered.

Rabbits which received injections of pure cultures of streptococci from the blood, postmortem material or stool of the patients, from a suspension of a patient's stool, from the peritoneal washings of mice which had received injections or from rabbits that had been given injections showed the same typical pathologic changes in their intestinal tracts, and from these areas the streptococci were recovered.

Organisms from rabbits which received injections of somewhat similar green-producing streptococci isolated from stools and other sources in patients free from any enteric infection were but slightly virulent, and in only a few instances did cultures produce intestinal lesions. These cultures were from the throats of two patients with acute pharyngitis and from the mastoid cells of two other patients with acute mastoiditis.

Streptococcus micro-apoikia enteritidis was not found in the intestinal tracts of normal rabbits studied at necropsy.

Convalescent rabbit serum protected rabbits against *Streptococcus micro-apoikia enteritidis.*

Rabbits remained normal when given injections of Berkefeld filtrates of cultures of streptococci recovered from patients with acute enteritis, of the blood purulent contents of the involved intestine or of peritoneal washings of mice that had been given injections.

Six macacus rhesus monkeys were given injections of streptococci recovered from patients with acute enteritis. The 6 monkeys became ill, and 5 died. All five showed typical lesions in the small intestines, and the streptococci were isolated from the contents of these areas. These reisolated streptococci produced the same lesions in the intestinal tracts of rabbits. Cultures of stools of the monkeys before injection did not contain these streptococci, but the organisms were present in cultures of the diarrheal stools after injection.

Generally, the first third of the small intestine of the rabbit or the major portion of the small intestine of the monkey appeared deeply injected. The lymphoid tissue in the wall of the involved portion of intestine was enlarged and purplish red. The contents of this portion of the intestine were blood-streaked, thick and purulent. The nodes in the mesentery, especially those adjacent to the intestine, were enlarged and deeply purple.

Microscopic study of sections of the small intestine revealed marked congestion in the mucosa, a polymorphonuclear and monocyte reaction in the mucosa and the submucosa and diplococci in the mucosa. Sections of mesenteric nodes revealed acute lymphadenitis and prominent germinal follicles with reticulo-endothelial hyperplasia in their centers. Sections of the spleen showed severe acute splenitis, congestion of the pulp with some hemorrhage and necrosis of the malpighian corpuscles and a polymorphonuclear reaction. The liver showed vacuolation of many of its cell nuclei. The kidneys gave the picture of a toxic nephrosis. Other tissues were normal.

BACTERIOPHAGE, Specific Treatment of Septic Infections, Particularly With the Aid of, Mac Neal, W. J. Am. J. M. Sc. **187**: 623, 1934.

The early specific diagnosis of infectious disease by immediate application of the appropriate bacteriologic technic is strongly urged.

Specific diagnosis facilitates proper care of the patient and isolation of the specific microbe makes possible the intelligent selection of specific serum or specific bacteriophage.

The appropriate application of antistreptococcus serum or of bacteriophages against the staphylococcus or the colon bacillus requires intimate collaboration of clinician and laboratory worker. The use of these agents is recommended as beneficial to some patients suffering from infection with the respective microbes.

NEUTROPENIAS, Effect of Leukocytic Cream Injections in the Treatment of, Strumia, M. M. Am. J. M. Sc. 187: 527, 1934.

Evidence is presented that injections of leucocytic cream intramuscularly in severe neutropenias is followed in most cases within one to four days, usually forty-eight hours, by an increase of mature granulocytic cells in circulation, along with a considerable clinical improvement.

The preparation of the leucocytic cream is as follows:

Withdraw the blood from the vein of the donor into citrate solution not over 150 c.c. Divide the blood in large tubes and centrifuge at high speed for thirty minutes. Carefully withdraw the clear supernatant plasma, part of which is kept for the preparation of leucocytic emulsion later. With a large mouth pipette and a rubber bulb remove carefully the buffy layer of leucocytes which has formed on the surface of the packed red cells. Do that for each tube and transfer the material to a Babcock cream tube. Add to this enough of the packed red cells to bring up well in the neck the surface of the fluid. Centrifuge again for twenty minutes at high speed. The leucocytes will now rise to the top. There are usually three well-defined layers: (1) clear plasma; (2) suspension of leucocytes and platelets; (3) leucocytes mixed with some red cells. This last layer has been found to be richer in polys, while the topmost layer contains more lymphocytes. Draw off with a large mouth capillary pipette this material, removing also the top layer of the packed erythrocytes. Suspend this material with plasma so as to make for each 100 c.c. of blood used from 5 to 10 c.c. of suspension. The material is used intramuscularly. As a rule, with high speed of the centrifuge the volume of the packed leucocytes is 1.6 per cent of the amount of blood used, and the suspension contains about ⅔ of the leucocytes present in the blood used. In smears of the suspension, the leucocytes appear very well preserved. It is desirable to check with a smear, or better, a cell count, that the suspension prepared is adequately rich in leucocytes. It is essential to separate the leucocytes from the blood as early as possible after the withdrawal of the blood from the donor. If for some reason the blood must be kept overnight, twice the amount of citrate should be used. The leucocytes thus prepared remain well preserved for a period of seven days at least, although the author never used them after five. Of course strict aseptic technic must be employed. Another important point is to remove as much of the plasma as possible after the first centrifugation and to have the topmost layer of the material transferred in the Babcock cream bottle as high as possible in the neck. If for some reason (deficiency of red cells) this is not possible, the surface of the fluid may be brought to the proper point by introducing in the Babcock cream bottle sterile glass beads. If the material necessary is ready at hand the whole procedure for 500 c.c. of blood can easily be accomplished in two hours or a little less.

The citrate solution, as anticoagulant, is prepared by dissolving 20 gm. of sodium citrate in enough sodium chlorid solution to make 100 c.c. The sodium chlorid solution percentage is 0.85. This solution contains for each c.c. 200 mg. of sodium citrate, which is sufficient to prevent coagulation of 50 c.c. of blood.

SICKLE CELL ANEMIA, Negative Results in the Treatment of, Diggs, L. W. Am. J. M. Sc. 187: 521, 1934.

Seven negro patients with sickle-cell anemia were treated with one or more of the following antianemic medications: whole liver, liver extract by mouth, liver extract subcutaneously, desiccated hog's stomach, bone marrow extract, iron, and transfusion. In 6 cases the erythrocyte, hemoglobin and reticulocyte values, and the clinical condition following therapy did not significantly change. In one negro child with the sickle-cell trait, observed during an initial anemic crisis associated with bronchopneumonia, clinical improvement and improvement of the anemia followed transfusion and the administration of liver and iron.

There is a dearth of information concerning the natural course of sickle-cell anemia in all its varied manifestations, and the possibility of spontaneous remissions must be considered in the evaluation of favorable therapeutic responses. The effectiveness of any form of therapy in the treatment of sickle-cell anemia remains to be proved.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor, Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

Electrocardiography*

THE student and practitioner of medicine in these days, if he is to be successful in his endeavor to understand the aspects and manifestations of disease, must be prepared to familiarize himself with the many innovations confronting him.

While the stethoscope and the percussing finger are still essential to the study of diseases of the heart, even the skilled physician must take cognizance of the rapid advances which have been made in the physiological field of cardiology, the practical application of which is found in the electrocardiogram.

No one will dissent from the author's prefatory statement that: "There is need for a treatise on electrocardiography, for use by the general practitioner, the medical student, and the specialist exclusive of the cardiologist, that they may learn the value of the electrocardiogram in the diagnosis of cardiac disease and learn to correlate the electrocardiographic findings with their clinical data. To meet that need this volume has been written."

The volume begins with a brief but excellent summary of the clinical concepts of heart disease and arrythmias, followed by a discussion of the conduction system. The principles and mechanism of the electrocardiograph are then clearly explained followed by a clear-cut discussion of the electrocardiogram. The remaining chapters are devoted to the clinical applications of electrocardiography illustrated by many graphs and their interpretation.

It is certain that to those who read this book with care electrocardiography will cease to be an esoteric subject and become a useful clinical aid.

There are few axioms more pertinent or which should be more often emphasized than that which closes the discussion of the clinical concepts of disease; namely: that whenever the cardiovascular system of an individual is under consideration there are four questions to be answered: (1) What caused the cardiac disease? (2) What is the structural lesion? (3) How is the physiology changed? and (4) What are his limitations?

This book may be recommended as presenting a very clear and practical discussion.

A Textbook of Histology†

THAT this textbook intended primarily for medical students has passed through six editions in eight years indicates its usefulness.

The present edition has been thoroughly revised and to a large extent rearranged and rewritten, particularly in the sections concerning the blood, the reticulo-endothelial system, the endocrine tissues, striped muscle, neuroglia, nervous tissue, the reproductive systems, and the lymphoid organs.

The final chapter (22 pages) presents an excellent discussion of histologic technic.

Both as a text for the student and as a work of reference this book is of use and value and for these purposes it may be thoroughly recommended.

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*Electrocardiography. By C. C. Maher, M.D., Assistant Professor of Medicine, Northwestern University. Cloth, pp. 250, 39 figures. William Wood and Co., Baltimore, Md.

†Textbook of Histology. By Harvey E. Jordan, Ph.D., Professor of Histology and Embryology, University of Virginia. Edition 6, cloth, pp. 738, 609 figures. D. Appleton-Century Co., New York.

Clinical Toxicology*

THE volume presents not only a comprehensive discussion of toxicology in general but incorporates an extensive discussion of industrial poisoning and a section on the prevention and compensation of industrial poisoning, by F. Koelsch, Director of the Bavarian Institute for Industrial Medicine, and a further section on the detection of poisoning, post-mortem, by K. Meixner, Professor of Forensic Medicine, Innsbruck.

While small, the book is comprehensive in scope and bears the stamp of thorough acquaintance with and wide experience in its field.

The references include material appearing as late as 1932, the American literature not being neglected although naturally the majority of the references are from European sources.

It will somewhat surprise the American reader to find drugs uniformly mentioned under trade names but, appreciating this, the translators have largely throughout the text translated these to a more familiar terminology and, in an appendix, have furnished a glossary.

The volume may be recommended as a useful and practical addition to the reference library on this subject.

A Laboratory Manual of Biological Chemistry†

WHILE primarily intended for the medical student, Folin's Manual for many years has been a useful reference text for the clinical laboratory and needs, therefore, no introduction.

The present fifth edition has been extensively revised to include all the modifications and new methods and now presents an up-to-date discussion of the subject.

The supplement, containing a detailed description of the methods used in the examination of urine and blood, will be of particular interest to the clinical laboratory worker because it presents important modifications of technic as well as new and hitherto unpublished data.

Among the new methods are a modification of the determination of blood creatinin, and three new micromethods for determinations on 0.1 or 0.2 c.c. of blood, and revision of the methods for the determination of sugar, nonprotein nitrogen, and uric acid.

These newer methods, yielding results somewhat at variance with older technics, are of importance in this respect and workers in this field must be familiar with them.

Of particular interest is the change in the preparation of blood filtrates for chemical analyses, this being prepared in this edition from unlaked blood, thus excluding the indiffusible, fixed materials of the red blood cells.

As before, this new edition of the manual will occupy the same distinguished position as its predecessors and may be highly recommended without reserve.

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*Clinical Toxicology: Modern Methods in the Diagnosis and Treatment of Poisoning. By Erich Leschke, Professor of Internal Medicine, University of Berlin. Translated by C. P. Stewart, Lecturer in General Biochemistry, University of Edinburgh and O. Durrer, Research Assistant to Professor Wieland, Munich. Cloth, pp. 346, 25 figures. William Wood & Co., Baltimore, Md.

†A Laboratory Manual of Biological Chemistry. By Otto Folin, Professor of Biological Chemistry, Harvard University. Edition 5. Cloth, pp. 368. D. Appleton-Century Co., New York.

The Journal of Laboratory and Clinical Medicine

VOL. 20 DECEMBER, 1934 NO. 3

Editor: WARREN T. VAUGHAN, M.D.
Richmond, Va.

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EDITORIALS

Bacon Was Right

BUFFETED as he is by the battledore and shuttlecock of opposing demands, the doctor's is surely one of the most paradoxical of all existences. The responsibilities thrust upon him by the nature of his duties certainly deserve, if they do not compel, opportunities for quiet, sober consideration—and yet his decisions, however momentous, must often be made upon the spur of the moment and from such facts as can be gathered rapidly under stress.

Although many an obscure and puzzling case really *demands* concentrated thought, the doctor's leisure, such as it is, is at the mercy of every hurried emergency. Many a battle against disease without doubt were best waged on a plan evolved after a council of war, so to speak, with leaders, experts, and authorities marshalled from the doctor's bookshelf, but no psychologist is required to determine the reaction of the patient requested to wait the formulation of advice until authorities had been consulted!

So, it would seem, the doctor's reading must often be done largely "on the run," which, perhaps, accounts for the somewhat sorry appearance of some

professional "libraries," too many of the volumes weak-kneed and broken-backed with age and, alas, somewhat frowsy from neglect.

The most common reply to this stricture—and one may rest assured that it is made more often than it is spoken, and has certainly occurred more than once to the intelligent patient—is that it is almost impossible to keep up with the flood of medical literature. To strengthen the alibi further it is often said —and with some truth—that medical literature is ephemeral literature; the up-to-the-minute "systems" of today are behind the times tomorrow; and the facts of one edition are the archaisms of the next.

To a large extent this is true, for while no one can deny that the two basic elements upon which the practice of medicine is founded—human beings and disease—are of undoubted antiquity, the angle of approach and the viewpoint from which they must be considered is constantly changing and undergoing modification, readjustment, and realignment in the light of changing concepts.

The problem in essence is to understand the mechanism involved and the results produced by the reaction of one variable, the individual patient, to another variable, the stimuli exerted by disease. And the understanding of either and both is constantly being revised, clarified, and augmented by a reconsideration of old concepts in the light of new facts, and by the formulation of new concepts arising from new observations which may or may not be justified, confirmed, or discarded by the cumulative evidence evolving from time and extended trial.

Thus it is that the ponderous, many-volumed sets of yesteryear are giving way to the more flexible publications capable of revision, and to the monographs which present in a single volume the current status of a single, circumscribed problem.

Even yet, however, there are difficulties, for the court of last resort is recorded experience. The experiments, the observations of today, must run the gauntlet of critical repetition and must stand or fall upon cumulative experience. No matter how promising they appear to be they cannot take their place in system, text, or monograph until a reasonable basis for their acceptance exists, and such reasonable basis arises from the recorded observations which accumulate in the various medical journals, each covering a more or less restricted field.

The medical textbook of tomorrow is, after all, really a digested critical summary of the medical literature as reported in the medical journals of which the number and variety groweth apace. Verily, the physician of good intention struggles in a flood of print—and who should say offhand which of it will join the steady stream of knowledge and which will pass as spray blown into nothingness by the winds of time and trial?

So it is often heard that there isn't time to read the medical journals; that they are too scientific and report too much scientific work (!); that they are not "practical" enough, and so on—and too often it is true that those who protest the loudest might benefit the most.

It would be impossible, of course, for anyone to read all the journals nor, indeed, is it worth while to read always all and every page of those he does

open—for no man can hope to know all of medicine as it is today. Every man must have some compelling interest, and there are few times, indeed, when there will not be something he may read with interest and profit.

Fortunately, in the abstract journals, there are available also concentrated surveys of the medical literature of the world which present briefly and succinctly the meat of lengthy contributions, the facts reduced to scientific syllogisms as it were, indicating almost laconically the basis for the ideas advanced. It is almost impossible to make this literary menu all-inclusive and keep within reasonable bounds; it must represent, therefore, the gist of the news.

Just what should be the basis for inclusion of an article? Some would have it entirely "practical"—but just what shall this mean, for who can tell in advance which research, which reported observation is practical and which abstract in the long run?

There can be no doubt of the practical value of the use of liver in pernicious anemia, yet this only came to pass because Whipple first studied the results of secondary anemia in dogs and a long time elapsed before the application of these purely abstract and scientific studies to the treatment of pernicious anemia in the human being was made by Minot and Murphy.

d'Herelle's studies in their inception were surely rather purely "scientific" and apparently only of somewhat academic interest, yet who can foretell the eventual relation of bacteriophage to the treatment of disease?

On the other hand, the "practical" observation of Dr. So-and-So upon the marvelous results of this or that method of treatment may be merely a question of conclusions based upon "one consecutive case"; or the result of imperfectly analyzed facts or imperfect or incomplete investigations.

It is not improbable that the main purpose of what sometimes passes for medical "literature" is to boost sales and that often it is read because it is so dogmatic and so certain and requires of the reader nothing but unquestioning acceptance of its statements.

The real purpose of scientific reading is twofold: to acquire information and to stimulate thought.

The periodical appearance of new "cures" for cancer in the public press is familiar to everyone—and how can the doctor answer the questions of his patients on such matters unless he has some knowledge of the present status of cancer research?

Unless he is to stagnate, content to fall behind and finally to become really ignorant of the things he should know, the doctor *must* read.

A library is only an asset when it is consulted and journals are of little use when stacked in dusty piles with the wrappers unbroken. If he must "read on the run" then let him read the abstract journals, and be it remembered that, whether their contents appear always and immediately "practical" or sometimes apparently merely abstractly scientific, they are always informative, always additions to one's general knowledge, and many times quite useful to have read. At least they will always furnish something to think about.

Bacon was right when he said: "Reading maketh a full man; conference a ready man; and writing an exact man; and, therefore, if a man write little he need have a great memory; if he confer little he need have a present wit; and if he read little, he had need have much cunning, to seem to know that he doth not."

—*R. A. K.*

Postoperative Wound Infections

WHILE the development of infection in originally clean surgical wounds is no longer the bugbear it was before the days of Pasteur and Lister, its occurrence is not yet a rara avis, and constitutes one of the most annoying and distressing complications of surgery.

Not only is it the immediate cause of prolongation of hospitalization and hence of increased expense, not to mention the suffering, anxiety, and loss of confidence in the surgeon with which it is often associated, but should the surgeon be so unfortunate as to encounter a virulent postoperative infection resulting in the death of the patient, the catastrophe is one almost impossible to explain to the satisfaction of all concerned.

When postoperative infections occur, therefore, and especially when they are more than very occasional in incidence, they are at once the subject of concern and discussion and the laboratory is often requested to inaugurate a sometimes extensive series of investigations.

It is, of course, quite natural and human under these circumstances to regard the operating room force with a questioning eye; to inquire as to the methods of sterilization in force; and to discuss the advisability of an extended bacteriologic survey. But the consensus of experience has shown that the first and essential factor in the survey is not the survey and study of minutia, but the survey of the problem as a whole.

In other words, for the successful management of such a problem it is essential to know, first of all, not merely that infection is present, but to know its *nature*. The first thing to be examined bacteriologically should be the wound. While this should be the invariable routine, it is especially important and may often be the determining factor, when postoperative infections occur in series, whether in the same service, in the same ward, or in a similar series of cases.

Without some definite plan of campaign the search is otherwise haphazard and even almost hopeless, for the possible avenues of infection are well-nigh legion.

The practical impossibility of sterilization of the skin throughout its entire thickness is well known; the metastatic invasion of surgical wounds from distant or cryptic foci is not unknown; and many accidental and even bizarre avenues may be thought of and even illustrated by experience.

But it is not the possibilities which should first be studied but the *probabilities*, and what the preeminent probabilities are can only be estimated when fundamental data are first collected.

It is, as a rule, of little use to begin with extensive culturing of operating room supplies, for it is rare indeed, where dressings, instruments, or solutions are sterilized with intelligence and understanding of the underlying principles that these are at fault, and commercial products are so safeguarded and controlled nowadays that infection from this source is practically unknown.

The place to begin is hence the wound. When infection occurs in series in clean appendectomies and the organisms recovered are predominantly of the colon group, the inferential probability is that their implantation occurred at the time of operation. When the organisms are predominantly of the Streptococcus group, the possibility arises that one or more of these concerned in the operations are carriers of this group; whereas, should the Staphylococci be in the main the offending organism, attention is directed toward the skin as a probable source of invasion.

When the nature of the infection is known the trend of the investigation becomes somewhat more obvious and, just as in the determination of the potability of water the survey of the source is of equal and sometimes even more importance than its bacteriologic study, so here the next step should be the survey of the operations. For in the vast majority of instances the underlying factor of paramount importance in postoperative wound infections is technic, and in its study keen observation is all that is required.

It is natural for surgeons to disclaim responsibility for infection but nevertheless, when it occurs, it is upon themselves and their methods that they should first focus attention. It is neither fair, just, nor invariably correct to place the burden at once upon the shoulders of the long-suffering assistant. If he be at fault—as sometimes he may be—the responsibility is still the surgeon's, not only for not training him properly by precept and example, but also for not detecting the error when it occurred.

The leaking of skin organisms from the sebaceous glands through a punctured glove is too obvious a source of infection to be overlooked, although this source is occasionally sometimes neglected; and the preoperative preparation of the skin is seldom at fault per se.

It is too often forgotten that bacterial invasion alone is not necessarily followed by infection; the tissues must be so prepared for infection as to permit the growth and reproduction of bacteria subsequent to invasion without which infection does not occur. The other factors often of determining importance, such as the numerical proportion of the bacterial invasion, etc., are, after all, of contributory importance.

In the survey of technic, therefore, attention should be directed to other factors of even greater importance.

Has anything occurred which would produce or contribute to devitalization of the tissues and so render them more liable to bacterial invasion? Has the operator or his assistant an "iron" or a "velvet" hand? Are the tissues manipulated *gently* or is pulling and tugging the order of the day? Are the gauze packs used to wall off the hyperemic and inflamed appendix, on removal dragged across the wound edges, in forgetfulness that if the intact intestinal wall is not impermeable to bacteria the diseased intestinal wall is still less so?

Was the wound dry when closed, or in the endeavor to make it so were relatively large masses of abdominal fat tied off with the vessels, later to necrose and form a fertile field for the skin organisms swept out from the relaxed skin of the perspiring patient?

These—and many more—are all of importance and may be determined without culture flasks or tubes. That they should be studied first of all is often indicated, as already suggested, by the original study of the nature of the infection.

It is well, of course, to survey methods of sterilization and so on, but it is better to survey operating technic for the answer will most often there be found.

Infection can be prevented—but its prevention requires cooperation, teamwork, and, strange as it may seem, not infrequently the legitimate use of a little imagination.

—*R. A. K.*

ITEM

The American Association for the Study of Goiter

The American Association for the Study of Goiter again offers the Van Meter Prize Award of $300 and two honorable mentions for the best essays on the subject of goiter provided they meet the standards of the award committee. The essays should be based on original research work on the subject of goiter, preferably its basic cause. The prize essay or its abridgment is to be presented at the annual meeting of the Association to be held in Salt Lake City, Utah, in June, 1935.

Competing manuscripts should be in the hands of the Corresponding Secretary, W. Blair Mosser, M.D., Kane, Pa., not later than April 1, 1935.

The first prize of $300 for the 1934 meeting was awarded to M. A. B. Brazier, Ph.D., B.Sc., London, England, for her essay, "The Impedance Angle Test for Thyrotoxicosis."

First honorable mention was awarded Prof. Ugo Cerletti, Genoa, Italy, for his essay, "Three Years of Experimental Research in the Etiology of Endemic Goitre."

Second honorable mention was awarded D. Roy McCullagh, M.D., Cleveland Clinic, Cleveland, Ohio, for his essay, "Studies in Blood Iodine Using a New Chemical Method."

Erratum

In the article by Drs. Graham and Randles in the October issue of the Journal, Fig. 1, which appears on page 91, is up-side down.

CORRESPONDENCE

Gauging the Dose of Insulin

THE article entitled "Gauging the Dose of Insulin," in the August issue of the JOURNAL OF LABORATORY AND CLINICAL MEDICINE, was of considerable interest because of the similarity in approach between Dr. Glassberg's calculations and those used by Dr. Henry Larson of our staff during the past year.

Approximately one year ago, Dr. Larson was interested in deducing the maximum dosage of insulin which could be safely given at one time to any diabetic, taking proper consideration of the weight and blood sugar of the patient. The formula derived does not have to be corrected by any factor such as is necessary with that of Dr. Glassberg.

According to Peters and Van Slyke, *Quantitative Clinical Chemistry*, Volume 1, 1932, page 82, the maximum amount of glucose in the body tissues is not over 50 grams, in a normal adult weighing 70 kilos. or 150 pounds. This represents a blood sugar of 100 mg. per cent. Since the total glucose in the body will vary directly as the weight of the body in pounds divided by three, and directly as the blood sugar, the following formula will represent the number of grams of glucose in any body:

A. $\left(\frac{BW}{3} \cdot \frac{BS}{100}\right)$ where BW represents weight in pounds
BS represents patient's blood sugar.

By subtracting $\frac{BW}{3}$ from Formula A, one arrives at the number of grams of excess glucose which have to be metabolized by the insulin administered:

B. $\left(\frac{BW}{3} \cdot \frac{BS}{100}\right) - \frac{BW}{3}$

Instead of dividing Formula B by 2, as is suggested in Dr. Glassberg's article, it would be safer to use the factor 2.5, since at its maximum efficiency, 1 unit of insulin can take care of 2.5 grams of glucose.
The formula then reduces to:

$$\frac{\left(\frac{BW}{300} \cdot \frac{BS}{}\right) - \frac{BW}{3}}{2.5} \text{ equals } \frac{BW\ (BS-100)}{750} \text{ equals total safe units of insulin.}$$

It is fully realized that the contraindications such as myocardial damage or coronary disease are to be scrupulously observed. As Dr. Glassberg remarks, diabetes cannot be treated merely by formula, since other factors, particularly concurrent infection, decrease the efficiency of insulin and require special consideration. Another point worth mentioning is the fact that insulin shock may result with a blood sugar above 100 mg. per cent; and that apparent comfort may exist with a blood sugar below 100 mg. per cent.

The physician may not wish to reduce the blood sugar to 100 mg. per cent. As a matter of fact, when one is dealing with diabetes of long standing, and the patient presents himself with a very high blood sugar, it might be more expedient to reduce it to 200 or 150 mg. per cent. The chronic diabetic feels more comfortable at such a level. In such a case, if one subtracts 2 or 1½ times $\frac{BW}{3}$ respectively, and divides the result by 2½, the quotient will represent the number of units of insulin required.

The above formulas are suggested merely as a guide for the use of insulin particularly in cases of real or imminent diabetic coma. In our hands it has proved safe and satisfactory, and the premise is based on the classic work of Peters and Van Slyke.

ATTILIO F. GALASSO, M.D.

OCTOBER 8, 1934
MORRISTOWN, NEW JERSEY